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Mucosal tears at the oesophagogastric junction (the Mallory-Weiss syndrome)

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SYNOPSIS This is a re-appraisal of the supposedly rare Mallory-Weiss syndrome in which 11 patients with mucosal tears at the oesophagogastric junction are described. The fact that these cases were collected from general hospitals within a short period suggests that the condition is more common than supposed and may account for a considerable proportion of the 20 to 25% of patients with upper gastrointestinal bleeding in whom no radiological abnormality can subsequently be found.

Of the 11 patients, eight presented with gastrointestinal bleeding, two with mediastinitis, and one without relevant symptoms. The classical history of antecedent vomiting before the bleeding was obtained in only four patients, its absence not excluding the diagnosis. The presence of a small hiatal hernia in four patients appeared to predispose to mucosal tears as did mucosal atrophy occurring with advancing age. Some experimental findings pertaining to the mechanism of the tears are presented.

In 1929, Mallory and Weiss described the syndrome which bears their name. Fifteen patients developed massive haematemesis after prolonged bouts of alcoholic debauch and in each case bloodless vomiting preceded the haematemesis. Post-mortem examinations of four of these patients revealed multiple mucosal fissures, 3-20 mm. in length and 2-3 mm. in breadth, situated around the circumference of the cardia with their long axes in line with the oesophagus. Histological examination revealed that these ulcers extended into the muscularis and their floor consisted of fresh fibrin and polymorphonuclear leucocytes. The appearances suggested an acute rupture of the mucosa and experiments on a stomach in a cadaver showed that similar lesions could be produced by gastric distension when the oesophagus was occluded at the level of the diaphragm. They concluded that 'pressure changes in the stomach during a disturbed mechanism of vomiting and continuous regurgitation of gastric juice over the cardia' were the important predisposing factors. In 1932, the same authors reported a further six cases of this condition, two of which were fatal. Post-mortem examination of the first of these revealed a single fissured ulcer extending into the muscularis. The second patient died of mediastinitis after rupture of an ulcer into the mediastinum. In only six of the total of 21 cases described by Mallory and Weiss was a tear actually seen and in the remainder the diagnosis was made on the history alone.

Decker, Zamcheck, and Mallory in 1953 recorded 11 cases of the Mallory-Weiss syndrome in approximately 11,000 necropsies over 20 years at the Boston City Hospital. In these cases the fissures ranged from 0·5 to 3 cm. in length and up to 0·7 cm. in width. Most fissures involved stomach and oesophagus, a few the stomach only, none the oesophagus alone. All lacerations were longitudinal. Six were single, five multiple; 10 occurred in males, one in a female. Five cases had evidence of chronic gastritis. In five cases the lesions were sectioned and appeared as flattened or trench-shaped ulcers based on the submucosa or muscularis. The walls and base showed extravasated red cells, leucocytes, and fibrin and in the older lesions granulation tissue had formed as well.

Kellogg and Blackburn (1954) reported three cases resembling the original description and Hardy (1956) made the diagnosis on gastroscopy in a young man whose haematemesis followed six hours after the vomiting of clear fluid occasioned by

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TABLE I

Case No.	*Sex*	*Age*	*Antecedent Vomiting*	*Gastrointestinal Bleeding*	*Hiatus Hernia Demonstrated*	*Duodenal Ulcer*	*Gastric Ulcer*	*No. of Tears*	*Associated Conditions*	*Diagnosis*	*Outcome*
1	F	74	−	+	+	−	−	1	Cholelithiasis	Laparotomy	Well, no further bleeding
2	F	83	+	+	−	−	+	1		Laparotomy	Well, no further bleeding
3	M	55	+	+	+	−	−	1		Gastroscopy	Well, no further bleeding
4	M	49	−	+	+	−	−	1	Chronic bronchitis	Gastroscopy	Well, no further bleeding
5	M	74	+	+	−	−	−	3	Cardiac infarction	Necropsy	Died
6	M	66	−	+	−	−	−	3		Necropsy	Died
7	M	59	−	+	−	−	−	2		Necropsy	Died
8	F	72	−	+	+	−	−	2	Acute pancreatitis	Necropsy	Died
9	F	75	−	−	−	−	−	1	Status asthmaticus	Necropsy	Died
10	F	84	−	−	−	+	−	1	Mediastinitis, (R) empyema	Necropsy	Died
11	M	75	+	−	−	+	−	3	Perforation of oesophagus, mediastinitis	Necropsy	Died

alcoholic excess. Kelley (1958) reported massive haemorrhage following gastroscopy from a tear 2 cm. in length situated 3 cm. below the oesophagogastric junction. He considered that this was a probable case of the Mallory-Weiss syndrome rather than a complication of the examination.

Small and Ellis (1958) reported a case treated surgically; a 44-year-old man vomited during the post-operative period after haemorrhoidectomy, and because bleeding continued laparotomy was performed and revealed a 3 cm. laceration at the cardia. This was sutured and pressure applied by a Sengstaken tube. Bleeding did not recur. Scott and Newton (1958) described a man aged 28 years with an eight-year history of psychogenic vomiting who, at operation for haematemesis, was found to have a single laceration 2·5 × 0·5 cm. which was sutured successfully. Shuttleworth and Hutt (1958) performed partial gastrectomy for haematemesis in a woman aged 53 with multiple small ulcers in a stomach which was the seat of a chronic gastritis. Post-operatively the patient continued to bleed and a total gastrectomy was performed. Five fissured ulcers were found in the cardia, 7-10 mm. × 1 mm., one of which had encroached on the oesophagus.

The present paper describes 11 cases of mucosal tears at the oesophagogastric junction and reports some experimental observations which have a bearing upon the mechanism by which these tears are produced.

CLINICAL FEATURES

The 11 patients (Table I) described in this paper were all shown to have mucosal tears at the oesophagogastric junction. The diagnosis was established at laparotomy (Cases 1 and 2), by gastroscopy (Cases 3 and 4), and at necropsy in the seven remaining cases. They comprised six men and five women whose ages ranged from 49 to 84, with a mean of 70 years.

Seven patients presented with haematemesis and/or melaena, and an eighth was admitted in a shocked state caused by bleeding into the stomach. The other three patients showed no signs of gastrointestinal haemorrhage, although at necropsy some altered blood was found in the stomach in one. Two of these patients developed mediastinitis but in the third the tear apparently caused no disability.

A history of vomiting preceding the haematemesis was obtained in only three of the eight patients with gastrointestinal bleeding and the time interval from the start of vomiting to the first haematemesis varied between half an hour and three hours. One of the three patients without gross gastrointestinal bleeding had vomited, making the overall incidence of antecedent vomiting in the series only four of the total 11 patients. Although an alcoholic debauch precipitated the initial vomiting on one occasion none of our patients were chronic alcoholics. Atrophic changes were noted at gastroscopy in one patient. One patient apparently sustained a mucosal tear at the cardia during a paroxysm of coughing, another when in status asthmaticus, and a third might have sustained the tear during an epileptic convulsion.

A small hiatus hernia was demonstrated radiologically in four patients and three of these gave a history of symptoms suggestive of gastro-oesophageal reflux.

Seven of the 11 patients succumbed, although death in two was not directly attributable to the tear. Of the eight patients who presented with gross haemorrhage, four recovered (two spontaneously and two after operation and suture), three died from

blood loss, and the other patient died of unrelated disease. Of the three patients who did not have gross bleeding, mediastinitis was the cause of death in two and the third died from an unrelated disease (status asthmaticus). Thus, the mucosal tear at the cardia was the cause of death in five of the 11 patients in the series.

PATHOLOGICAL FINDINGS

The cardio-oesophageal lesions in those patients who came to necropsy (Table I) all presented similar macroscopic and microscopic features, all of which were comparable with those found in previous descriptions of the ulcers associated with the Mallory-Weiss syndrome. With the exception of the two patients in whom the oesophagus had perforated, the ulcers were superficial and oval or linear, with the long axis in line with the oesophagus, or else pear shaped, with the acute angle directed towards the stomach (Fig. 1). The size of the ulcers varied from 3·5 × 1·4 cm. to 1·2 × 0·1 cm. and their number from one to three. The bases of the ulcers were covered by blood or yellow slough; the edges had steep sides with some heaping up of the surrounding mucosa.

The microscopic appearance was that of acutely inflamed ulcers which had penetrated the muscularis mucosa (Fig. 2). The base of the deepest ulcer rested on the oblique muscle, but most were limited to the submucosa. The craters contained a fibrinopurulent exudate with a variable quantity of blood, and the floor was oedematous and acutely inflamed. A certain amount of fibrin had been deposited, but no fibrous tissue appeared to have been formed. A plexus of large, thin-walled blood vessels was usually found in the submucosal tissure surrounding the ulcers (Fig. 2).

The wall of the oesophagus in the upper portion of the tear had perforated in two patients (Fig. 3) and caused death from mediastinitis.

CASE REPORTS

CASE 1 A woman, aged 74 years, was admitted following a brisk haematemesis. The vomit was bright red blood which welled up effortlessly. For two years she had suffered from mild heartburn occurring 10 minutes after meals and relieved by food and alkalis. Eighteen months before the present admission a barium meal had revealed a small hiatus hernia with free reflux.

On admission her blood pressure was 90/55 mm. Hg and the haemoglobin was 57% (8·4 g.%). Abdominal examination showed an aortic aneurysm, but nothing else of note. A 'ward' barium meal (Cantwell, 1960) revealed gall-stones but no lesion in oesophagus, stomach, or duodenum.

Bleeding continued and three days after admission operation was performed by one of us (B.G.P.). The whole gut was full of blood. There was a saccular aneurysm extending from the upper abdominal aorta to both iliac arteries and the presence of gall-stones was confirmed. The stomach and duodenum felt normal and gastrotomy was performed. There was much fresh blood in the fundus and on the posterior wall of the cardia there was a narrow elongated fissure approximately 2 × 0·2 cm. which was seen to be bleeding freely. Two catgut sutures applied to the fissure controlled the bleeding. Recovery was uneventful.

CASE 2 A woman, aged 83 years, complained of intermittent pain in the left hypochondrium of three years' duration. The pain was unrelated to meals and had never been accompanied by vomiting. On the day of admission she vomited a small amount of clear fluid followed half an hour later by a large haematemesis. She continued to bleed and her condition deteriorated in spite of blood transfusion.

At laparotomy by one of us (R.E.W.), 24 hours after admission, the stomach was dilated with blood and clot but there was no external evidence of a gastric or duodenal ulcer. A wide gastrotomy was performed. A small scar was found on the lesser curvature but this was not the source of bleeding. There was a tear about 2 cm. long on the anterior wall of the stomach immediately below the oesophagogastric mucosal junction. This tear was sutured with catgut and the patient made an uneventful recovery.

CASE 3 A bricklayer, aged 55 years, had suffered from intermittent heartburn after meals and on bending for 10 years. On the day of admission, after several pints of beer, he vomited twice. Feeling better again, he had a further drink which precipitated further vomiting, this time productive of blood.

On admission to hospital one hour later he was rather pale but showed no signs of shock. No physical abnormality was found. The haemoglobin was 78% (11·5 g.%) and stool occult blood tests were strongly positive during the first week in hospital. Barium studies three days after admission to hospital revealed no radiological abnormality of the oesophagus, stomach, or duodenum. Gastroscopy seven days after the patient's coming into hospital revealed an atrophic gastric mucosa in which mucosal blood vessels could be seen. The antrum appeared normal and showed active peristalsis. At the cardia, however, a shallow linear ulcer with a greyish white base and reddened margins extended from the anterior wall of the stomach across the mucosal junction into the oesophagus (Fig. 4).

Recovery was entirely uneventful and he was discharged from hospital after 14 days, quite free from symptoms. A further barium meal two months after discharge revealed a small hiatus hernia.

CASE 4 A 49-year-old van driver had suffered from bronchitis for several years. On the day before admission to hospital, after a bout of coughing, a clot of blood appeared in the mouth and shortly afterwards he passed

FIG. 1. *Case 5. Necropsy specimen showing three tears at the cardia. All extend across the mucosal junction and penetrate to the submucosa.*

FIG. 2. *Histological section of the largest tear in Fig. 1 (×35). This has been converted into an acutely inflamed ulcer. Note the proximity of the tear to the blood vessels of the submucosa.*

FIG. 3. *Case 11. Stomach and oesophagus at necropsy showing three tears (marked with arrows), one of which has perforated the oesophagus.*

FIG. 1

FIG. 3

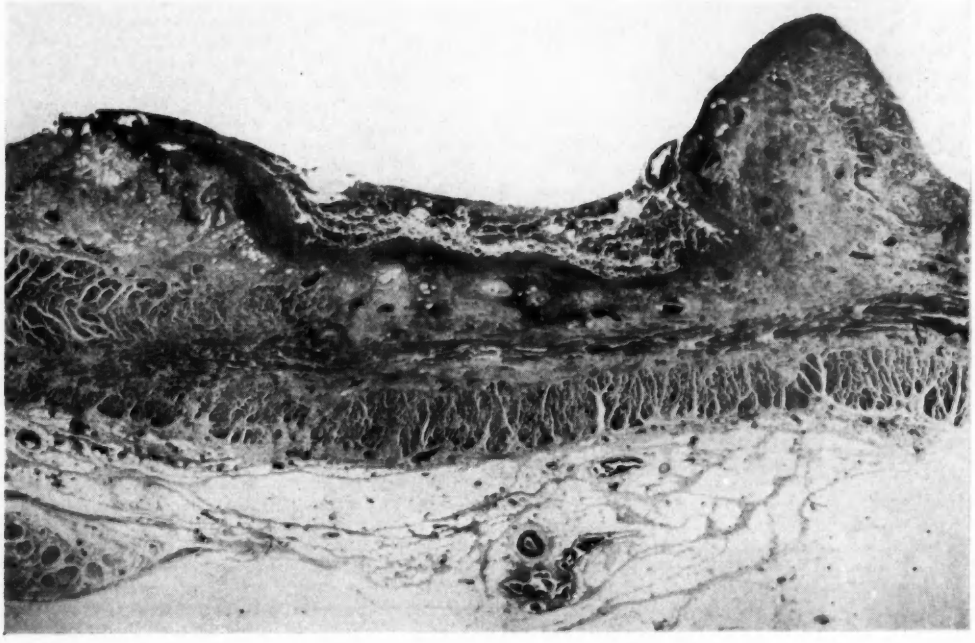

FIG. 2

FIG. 5

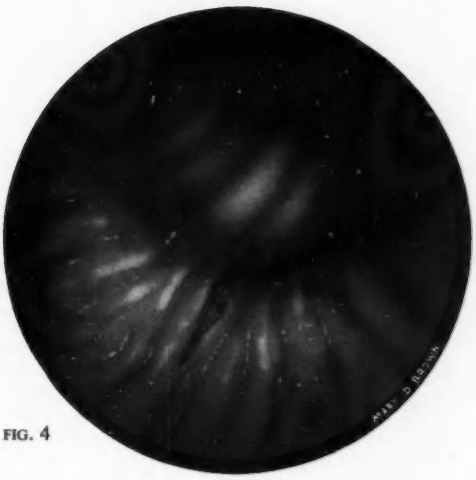

FIG. 4

FIGS. 4 and 5. *Gastroscopic appearances in Cases 3 and 4 showing a healing tear extending across the oesophagogastric junction (Fig. 4) and a superficial linear ulcer at the cardia with some blood clot adherent to its central portion (Fig. 5).*

a melaena stool. On the day of admission he fainted at work and passed several more melaena stools.

Examination on admission revealed pallor but no other abnormality. He was transfused with 3 pints of blood. A ward barium meal showed a small hiatus hernia but no other abnormality. Gastroscopy two days after admission revealed a linear ulcer on a reddened mucosal fold on the anterior aspect of the cardia (Fig. 5). This appeared to extend into the oesophagus and old blood clot was adherent to its central portion. The gastric mucosa looked slightly atrophic but no other abnormality was found. Bleeding ceased spontaneously and he was discharged 10 days later.

CASE 5 A man, aged 74 years, was admitted with haematemesis. Two weeks before admission he experienced sudden chest pain when walking uphill. This pain subsided with rest, but recurred frequently until the day of admission when he developed severe precordial pain and vomited twice. The material vomited on the second occasion contained a little blood. Shortly after this he had a haematemesis of approximately 2 pints.

On admission his general condition was poor, he was grossly anaemic and electrocardiography showed the changes of anterior myocardial infarction. Blood transfusion was started but he died suddenly 36 hours after admission following another haematemesis.

Post-mortem examination showed severe coronary atheroma with a recent small anterior myocardial infarct. Blood was found in the stomach, small and large bowel. Three fissured ulcers were present at the cardia of the stomach, the largest measuring 3·5 × 1·5 cm. (Fig. 1). The microscopic appearance was that of an acutely inflamed ulcer penetrating to the muscularis mucosa (Fig. 2). No other lesion was found in the gastrointestinal tract.

CASE 6 A man, aged 66 years, who had suffered from epilepsy for 10 years, was admitted unconscious; he was severely shocked and died before reaching the ward. He had never suffered from dyspepsia and there had been no recent vomiting or overt gastrointestinal bleeding.

At necropsy the stomach and upper jejunum were distended by several pints of blood. There were three fissured ulcers in the cardiac region measuring 2·5 × 0·75 cm., 1·5 × 0·5 cm., and 1·0 × 0·5 cm. respectively. One involved stomach and oesophagus while the other two were confined to the stomach. Fresh clot was adherent to the floor of one ulcer, suggesting that bleeding had stopped before death. The gastric mucosa was normal and no other lesion was found in the gastrointestinal tract. Old infarcts were present in the brain associated with cerebral arteriosclerosis and these probably accounted for the epilepsy.

Histological sections of the oesophagogastric lesions showed a breach of the mucosa exposing the vascular submucosa but not extending deeper into the wall. Laminated blood clot was adherent to the floor of one ulcer (Fig. 6). There was very little associated cellular infiltration of the ulcers and most of the cells were polymorphs, indicating the acute nature of the lesion. The gastric mucosa was healthy with no evidence of gastritis.

CASE 7 A man, aged 59 years, was admitted with a history of having passed several small black motions the same day. There had been no haematemesis, previous dyspepsia, or vomiting. On admission his general condition was good with a haemoglobin level of 83%

FIG. 6. *Case 6. Histological section of mucosal tear showing adherent laminated blood clot. There is little surrounding cellular infiltration.*

(12·3 g. %). Recurrent haematemeses during the next two days necessitated blood transfusion and led to the decision to operate. However, before he could be transferred to the operating theatre he died suddenly after a massive haematemesis.

At necropsy the stomach and upper intestine contained large amounts of blood. The sources of bleeding were two linear mucosal tears in the cardiac region. These measured 2·75 × 1·25 cm. and 1·0 × 0·75 cm. The first lay across the oesophagogastric junction and the second was confined to the stomach (Fig. 7). No other gastrointestinal source of bleeding was found.

CASE 8 A woman, aged 72 years, was admitted in a confused state having had a haematemesis. She had vomited after meals for some time but the last such episode had been weeks previously. A barium meal showed a small hiatus hernia but no other abnormality in the oesophagus, stomach, or duodenum. Her condition gradually deteriorated and she died one week later without further bleeding.

Necropsy showed that the cause of death was cerebral softening due to generalized arteriosclerosis. There was resolving acute pancreatitis and above the oesophagogastric junction were two healing tears. A small hiatus hernia was present but no other abnormality was found in the gastrointestinal tract.

CASE 9 A woman, aged 75 years, who had suffered from asthma for 10 years, was admitted in status asthmaticus. Her condition deteriorated and she died five days later. There was no vomiting before death.

Necropsy showed a small mucosal tear, 1·5 × 0·7 cm., lying across the oesophagogastric mucosal junction. There was no evidence of bleeding and no other lesion was present in the gastrointestinal tract.

CASE 10 A woman, aged 84 years, died following a fracture of the neck of the femur. There was no history of vomiting or haematemesis before death. At necropsy, a tear measuring 4·6 × 1·0 cm. was found, mainly in the stomach but extending across the oesophagogastric mucosal junction (Fig. 8). This had perforated at its upper end causing mediastinitis and a right-sided empyema. A chronic duodenal ulcer was also found. No evidence of haemorrhage was discovered.

CASE 11 A man, aged 75 years, had suffered from a chronic duodenal ulcer for 40 years, and often vomited.

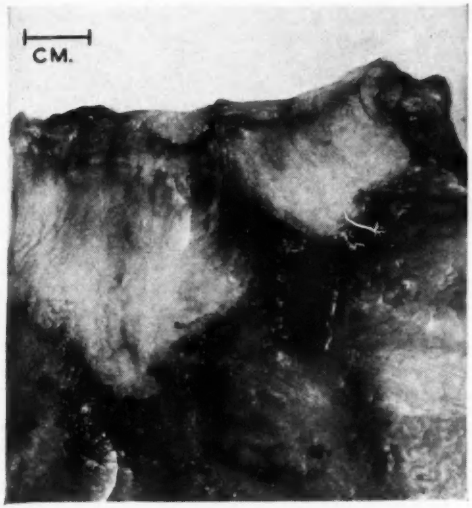

FIG. 7. *Oesophagogastric junction in Case 5 showing two tears.*

FIG. 8. *Oesophagogastric junction in Case 10. The oesophagus had perforated at the upper end of the tear causing mediastinitis.*

He was admitted complaining of sudden severe generalized abdominal pain accompanied by frequent vomiting, but no haematemesis. The pain later localized in the left hypochondrium and lower left thorax. His condition rapidly deteriorated and he died 15 hours later.

At necropsy, three linear tears were found, measuring 5 × 0·8 cm., 2·5 × 0·7 cm., and 1·6 × 0·7 cm. All crossed the oesophagogastric mucosal junction and the largest had perforated the wall of the oesophagus (Fig. 3). A posterior mediastinitis and bilateral pleural effusions were found. The stomach contained altered blood and a chronic duodenal ulcer was present.

EXPERIMENTAL OBSERVATIONS ON THE MECHANISM OF MUCOSAL TEARS AT THE CARDIA

To investigate the mechanism of the production of tears at the oesophagogastric junction and to obtain some measure of the magnitude of the forces involved, experiments were made in the cadaver within 36 hours of death. These were designed to determine the intragastric pressure required to produce mucosal tears of the stomach *in situ*. For comparative purposes measurements of intragastric and intraoesophageal pressure during straining and retching were made in five normal subjects.

NECROPSY STUDIES Through a window in the thoracic cage the oesophagus was clamped at the level of the arch of the aorta taking care not to disturb the anatomy of the diaphragm or its attachments. By reflecting the left lung forward the lower oesophagus could be inspected continuously throughout the subsequent distention. Through an incision in the anterior abdominal wall the stomach was transected at the antrum and the cardiac region inspected to exclude antemortem mucosal tears. A wide-bore brass cannula, with a side arm to which was attached a mercury manometer, was then tied into the transected proximal portion of the stomach and through this the organ was distended with air. Intragastric pressure was increased further by manual pressure on the anterior wall of the already distended stomach until a predetermined level was reached. This was maintained momentarily and then released. The cannula was removed and the cardia inspected for tears. The results obtained are shown in Table II, where it will be seen that the

TABLE II

INTRAGASTRIC PRESSURE REQUIRED TO PRODUCE MUCOSAL TEARS AT THE CARDIA IN THE CADAVER

Intragastric Pressure (mm. Hg)	*No. of Cadavers*	*No. in which Tears were Produced*
90	5	0
100	5	1
110	5	0
120	6	0
130	5	2
140	5	3
150	6	6

frequency with which mucosal tears were produced was directly related to the pressure applied; a tear was

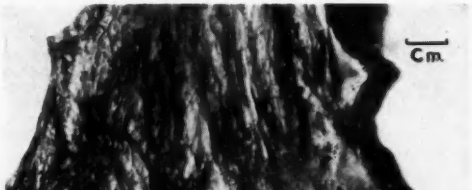

FIG. 9. *Three tears (marked by arrows) produced by distending the stomach* in situ *at necropsy. These bear a close resemblance in position and shape to antemortem lacerations. Submucosal vessels are visible in the bases of the tears.*

produced in one cadaver with an intragastric pressure of 100 mm. Hg and at 150 mm. Hg tears were produced in each of six cadavers. The tears were longitudinal in position and were confined to the oesophagogastric junction (Fig. 9), closely resembling those described in the preceding case histories. Histological section of these experimental tears of course showed no vital reaction but often revealed the submucosal vessels to be exposed (Fig. 10).

STUDIES DURING STRAINING AND RETCHING Intragastric pressure was recorded from the stomach just below the cardia, through a fine, water-filled polyethylene tube attached to a capacitance manometer outside the body, in five healthy staff and students. With the subject fasting and lying on the left side pressures were measured during straining and during retching induced by touching the back of the throat with a wooden spatula. The level of intragastric pressure reached varied from 120 to 160 mm. Hg and this was usually sustained for several seconds. Transient increases of pressure of up to 200 mm. Hg were found.

Intra-oesophageal pressure rose to levels of 35 to 50 mm. Hg in the five subjects, indicating that during straining intrathoracic pressure is considerably less than intragastric pressure.

DISCUSSION

Mallory and Weiss ascribed the mucosal lesions they noted to laceration caused by 'pressure changes in the stomach during a disturbed mechanism of vomiting'. Although this view has in general been

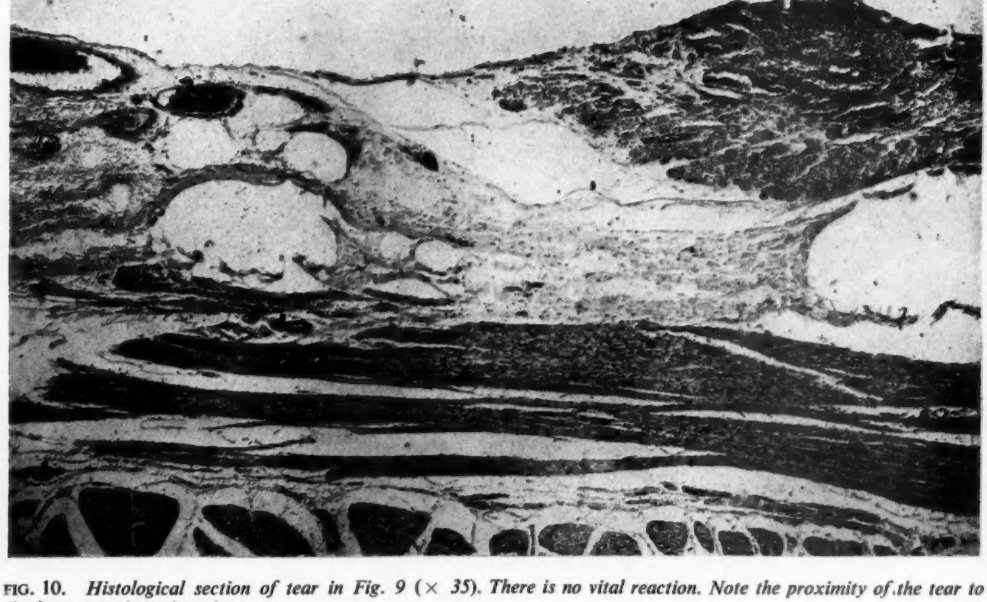

FIG. 10. *Histological section of tear in Fig. 9 (× 35). There is no vital reaction. Note the proximity of the tear to the large vessels in the submucosa.*

accepted, Bruce and Dudley (1959) have suggested that the mucosal lesions start as linear acute ulcers rather than as tears in the mucosa. These they would regard as a variety of proximal peptic ulcer of the type described by Dieulafoy (1898) and by Gilchrist and Chun (1954). We believe that these lesions begin as tears which are subsequently converted into shallow ulcers before undergoing complete healing. This view is based on the fissured shape of the lesions and the fact that similar tears can be produced by distending the stomach of the cadaver.

Tearing of the gastric mucosa will presumably depend upon the degree of stretching of the stomach, which will be related to the pressure gradient across the wall rather than the absolute level of intragastric pressure. During straining, retching, and vomiting the increase in intragastric pressure is largely due to increase in intra-abdominal tension brought about by contraction of the abdominal wall muscles and the diaphragm. Hence the degree of stretching of the stomach wall would be much less than that produced by gastric distension in the cadaver in spite of the fact that we recorded comparable levels of intragastric pressure. However, mucosal tears are found almost invariably at the oesophagogastric junction and seem to occur more frequently in patients with hiatus hernia. In fact a hiatus hernia was demonstrated in four of our 11 patients. In hiatus hernia the intraluminal pressure in the herniated portion of the stomach corresponds with that in the subdiaphragmatic portion of the organ rather than with the general level of intrathoracic pressure (Atkinson, Edwards, Honour, and Rowlands, 1957). Under these circumstances, the pressure gradient across the gut wall at the oesophagogastric junction will be high because intrathoracic pressure during straining, retching, and vomiting is much lower than intra-abdominal pressure. The intra-oesophageal pressures of up to 50 mm. Hg which we recorded from normal subjects during retching and straining give an approximate measure of intrathoracic pressure. Hence, if the oesophagogastric junction lies in the thorax the pressure gradient across its wall will be of the order of 100 mm. Hg or more, and stretching in this region will be greater than in the wall of the intra-abdominal portion of the stomach.

Not in every patient who sustains a mucosal tear at the cardia can a hiatus hernia subsequently be demonstrated. As the diagnosis of small hiatus hernias is often difficult both at radiological examination and at necropsy, it may be that all our patients did in fact suffer from this abnormality. An alternative explanation would be that the sharp descent of the diaphragm during vomiting does in fact cause the cardia to lie temporarily in the thorax, and it is at this time that the tears may be sustained. Nauta (1956) has demonstrated considerable movement of the oesophagogastric junction in relationship to the diaphragmatic hiatus in the intact dog and such mobility could provide an explanation for the causation and localization of mucosal tears at the cardia in the human. Fleischner (1956) believes that mucosal tears at the cardia occur when the oesophagogastric junction is in the thorax and thus that they are produced by a mechanism analogous to that by which oesophageal rupture is caused (Mackler, 1952). Indeed in two of our cases (10 and 11), with typical tears, the oesophagus was perforated.

An additional factor which requires consideration is the resistance of the mucosa. Gastric mucosal atrophy was present in five of the 11 cases described by Decker *et al.* (1953) in Shuttleworth's and Hutt's patient and in at least one patient of the present series (Case 2). The aetiological significance of this is difficult to assess as the condition is so common (Joske, Finckh, and Wood, 1955), but mucosal atrophy occurring with age may well predispose to mucosal tears, and the fact that most of our patients were advanced in years would be in keeping with this view. Chronic alcoholism, which features so prominently in the original description of the syndrome, was not present in any of our patients.

Mucosal tears at the oesophagogastric junction are generally regarded as a rare cause of gastrointestinal bleeding and receive little, if any, mention in most of the reports of large series of patients with upper gastrointestinal haemorrhage. However, Chalmers, Zamcheck, Curtins, and White (1952) mention three cases of the Mallory-Weiss syndrome in a series of 101 patients, and Palmer (1952) suspected this diagnosis on clinical grounds in seven of 121 patients admitted with gastrointestinal haemorrhage, although visual confirmation was not obtained. Our own experience suggests that mucosal tears account for a significant proportion of upper gastrointestinal bleeding episodes; after our attention had been drawn to this disorder by Case 1, no fewer than four (Cases 2, 3, 4, and 5) of the next 73 consecutive patients admitted with haematemesis and/or melaena were found to have mucosal tears in the region of the cardia. It is generally agreed that no cause can be found for upper gastrointestinal bleeding in a considerable proportion of patients admitted to hospital on this account; this proportion amounted to 26% of 325 patients in Schiff's series (1947) and 21% of 1,910 patients reported by Avery Jones (1956). Although the diagnosis in these patients has been presumed to be acute ulceration, it seems probable that a number may in fact have suffered from mucosal tears at the cardia.

Emesis followed within a few hours by haematemesis is the classical clinical presentation. Vomiting from almost any cause may be responsible for the initial episode and alcoholic excess (Case 2), pancreatitis (Case 8), peptic ulcer, which was present in three of our cases (2, 10, and 11), uraemia (Kellogg and Blackburn, 1954), and vomiting of pregnancy have all been incriminated. However, in no fewer than seven of our patients no history of vomiting preceding the gastrointestinal bleeding could be obtained. Violent contraction of the diaphragm and abdominal muscles during paroxysms of coughing, status asthmaticus, and an epileptic convulsion might have been the cause of the mucosal tears in three of our patients.

It seems probable that not every mucosal tear is followed by gastrointestinal haemorrhage. This was illustrated by Cases 9 and 10 in our series in which mucosal tears were found at necropsy in patients who had never had overt bleeding. If no haemorrhage occurs these tears will almost certainly pass unnoticed unless the lesion involves the full thickness of the wall causing mediastinitis (as in patients 10 and 11).

The diagnosis of mucosal tears at the cardia presents many difficulties even when the clinician is alert to the possibility. These lesions cannot be demonstrated radiologically, they are in a region which is difficult to inspect endoscopically, and will be missed at laparotomy unless an extensive gastrotomy be performed. Furthermore, they may be mistaken for artefacts by the endoscopist, by the surgeon at operation, or by the pathologist at necropsy. Bleeding from these tears may cease spontaneously, and if the diagnosis is suspected on clinical grounds endoscopy may provide confirmation if performed within a week of bleeding.

Occasionally bleeding continues and laparotomy may have to be undertaken as in Cases 1 and 2 of this series. Here again the diagnosis is largely dependent upon the surgeon bearing in mind the possibility of mucosal tears; in the absence of an obvious source of bleeding the cardia should be inspected through a gastrotomy opening. Should such an inspection be neglected and an empirical 'blind' gastrectomy be performed, bleeding may well continue. If a mucosal tear is found at operation carefully placed sutures will usually control the bleeding (Scott and Newton, 1958) as in Cases 1 and 2.

SUMMARY

Eleven patients with mucosal tears at the oesophagogastric junction are reported, the diagnosis being established by gastroscopy in two, at laparotomy in two, and at necropsy in the remaining seven. Gastrointestinal bleeding was the presenting feature in eight and two of them required laparotomy for control of haemorrhage. Of the three remaining patients, two suffered from mediastinitis and the third had no relevant symptoms. It is suggested that these mucosal tears account for a significant proportion of the 20-25% of haematemeses in which no cause can be discovered.

Similar mucosal tears could be produced at the cardia in the cadaver by distending the stomach to a pressure of 100-150 mm. Hg.

Four of our patients suffered from small hiatus hernias and it is suggested that these mucosal tears occur when the cardia is displaced into the thorax and the pressure gradient across the wall is high.

We are indebted to the physicians and surgeons under whose care some of these patients were admitted to hospital, to Miss Mary D. Brown, of the Department of Surgery, The General Infirmary at Leeds, for the paintings of the gastroscopic appearances in Cases 3 and 4, and to Mr. F. P. Hainsworth, F.R.P.S., A.I.B.P., of St. James's Hospital, for the photographs.

REFERENCES

Atkinson, M., Edwards, D. A. W., Honour, A. J., and Rowlands, E. N. (1957). The oesophagogastric sphincter in hiatus hernia. *Lancet*, **2**, 1138-1142.

Bruce, J., and Dudley, H. A. F. (1959). Gastrectomy for massive gastrointestinal haemorrhage of unknown cause. *Ibid.*, **2**, 992-994.

Cantwell, D. F. (1960). Ward barium meal examination in acute gastro-intestinal haemorrhage. *Clin. Radiol.*, **11**, 60-64.

Chalmers, T. C., Zamcheck, N., Curtins, G. W., and White, F. W. (1952). Fatal gastrointestinal hemorrhage: clinico-pathologic correlations in 101 patients. *Amer. J. clin. Path.*, **22**, 634-645.

Decker, J. P., Zamcheck, N., and Mallory, G. K. (1953). Mallory-Weiss syndrome. Hemorrhage from gastroesophageal lacerations at the cardiac orifice of the stomach. *New Engl. J. Med.*, **249**, 957-963.

Dieulafoy, G. (1898). Exulceratio simplex: L'intervention chirurgicale dans les hématémèses foudroyantes consécutives a l'exulcération simple de l'estomac. *Bull. Acad. Méd. (Paris)*, 3 ser., **39**, 49-84.

Fleischner, F. G. (1956). Hiatal hernia complex. Hiatal hernia, peptic esophagitis, Mallory-Weiss syndrome, hemorrhage and anemia, and marginal esophagogastric ulcer. *J. Amer. med. Ass.*, **162**, 183-191.

Gilchrist, R. K., and Chun, N. (1954). Severe hemorrhage in presumed peptic ulcer. Surgical treatment in the absence of demonstrable lesion. *A.M.A. Arch. Surg.*, **69**, 366-377.

Hardy, J. T. (1956). Mallory-Weiss Syndrome. Report of case diagnosed by gastroscopy. *Gastroenterology*, **30**, 681-685.

Jones, F. Avery (1956). Hematemesis and melena with special reference to causation and to the factors influencing the mortality from bleeding peptic ulcers. *Ibid.*, **30**, 166-190.

Joske, R. A., Finckh, E. S., and Wood, I. J. (1955). Gastric biopsy. A study of 1,000 consecutive successful gastric biopsies. *Quart. J. Med.*, **24** (n.s.), 269-294.

Kelley, M. L., Jr. (1958). Massive hemorrhage following gastroscopy; probable example of Mallory-Weiss syndrome. *Amer. J. dig. Dis.*, n.s. **3**, 454-463.

Kellogg, R. O., and Blackburn, N. P. (1954). The Mallory-Weiss syndrome. *J. Maine med. Ass.*, **45**, 318-320.

Mackler, S. A. (1952). Spontaneous rupture of the esophagus: an experimental and clinical study. *Surg. Gynec. Obstet.*, **95**, 345-356.

Mallory, G. K., and Weiss, S. (1929). Hemorrhages from lacerations of the cardiac orifice of the stomach due to vomiting. *Amer. J. med. Sci.*, **178**, 506-515.

Nauta, J. (1956). The closing mechanism between the oesophagus and the stomach. *Gastroenterologia* (*Basel*), **86**, 219-232.

Palmer, E. D. (1952). Observations on the vigorous diagnostic approach to severe upper gastrointestinal hemorrhage. *Ann. intern. Med.*, **36**, 1484-1491.

Schiff, L. (1947). In *Signs and Symptoms*, ed. C. M. MacBryde, pp. 344-359. Lippincott, Philadelphia.

Scott, N. M. Jr., and Newton, D. E. (1958). Mallory-Weiss syndrome—report of a case treated surgically. *Amer. J. dig. Dis.*, n.s. **3**, 464-468.

Shuttleworth, K. E. D., and Hutt, M. S. R. (1958). Mallory-Weiss syndrome—a case with recovery after total gastrectomy. *Brit. J. Surg.*, **46**, 1-3.

Small, A. B., and Ellis, P. R. (1958). Laceration of the distal esophagus due to vomiting (the Mallory-Weiss syndrome). Report of a case with massive hemorrhage and recovery after repair of the laceration. *New Engl. J. Med.*, **258**, 285-286.

Gut, 1961, 2, 12

Islet tumours of the pancreas with intractable diarrhoea

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SYNOPSIS This paper reports the association between severe, prolonged, watery diarrhoea and islet cell tumours of the pancreas. There appear to be significant differences between patients with these symptoms and those with the classical Zollinger-Ellison syndrome.

The association of non-insulin-secreting islet cell tumours of the pancreas with intractable peptic ulceration has been well documented by Zollinger and Ellison (1955) and by Ellison (1956). In their first communication (1955) they reported two such patients, both of whom presented with chronic ulceration of the jejunum. In each patient various surgical procedures, normally judged sufficient to control peptic ulceration, failed to prevent early recurrence. Necropsy in the first revealed a pancreatic islet cell tumour, whereas in the second, at a fifth abdominal exploration for recurrent gastrointestinal ulceration, routine inspection of the abdominal viscera disclosed two nodules in the pancreas; histological examination showed them to be composed of cells other than the B type. In neither patient was there evidence of hyperinsulinism, though in one a glucose tolerance test showed a slight defect.

Since 1955 many more examples of the Zollinger-Ellison syndrome have been reported (Summerskill, 1959; Pender, 1959; Rawson, England, Gillam, French, and Stammers, 1960). Indeed, by 1959 Zollinger and Elliott had collected a total series of 75 patients. The main features of this syndrome are intractable peptic ulceration of the stomach, duodenum, or jejunum, rapid recurrence of ulceration after apparently adequate surgery, a high level of gastric secretion, hypertrophic gastritis, and a non-insulin-secreting islet cell tumour of the pancreas, more commonly malignant than benign. More recently steatorrhoea has been noted (Summerskill, 1959; Maynard and Point, 1958; Rawson *et al.*, 1960).

The first patient described by Zollinger and Ellison also complained of intractable diarrhoea which had begun eight years before the diagnosis of peptic ulceration; the absence of pathogens in the stools, together with the failure of oral antibiotics to control the diarrhoea, lead to an erroneous diagnosis of spastic colitis. Unfortunately this case report does not comment on the further course of the diarrhoea throughout the remaining two years of life which followed the diagnosis of jejunal ulceration; in fact little attention seems to have been paid to this diarrhoea at any point, although it was the patient's main disability. Forty and Barrett (1952) had previously described a patient with multiple islet cell tumours of the pancreas in whom persistent diarrhoea developed one year before peptic ulceration. Priest and Alexander (1957) reported a patient who, four years before coming under their care, had had a tumour of the body and tail of the pancreas removed. She remained well after this until jaundice developed for which cholecystectomy was performed. In the following year she developed watery diarrhoea; at necropsy several months later an islet cell tumour of the pancreas was found. In this patient there was no history or evidence of peptic ulceration until she was treated by cortisone, after which she developed melaena. Verner and Morrison (1958) described two more patients in whom severe intractable diarrhoea had developed; in each an islet cell tumour of the pancreas was found at necropsy, but in neither was there evidence of associated gastrointestinal ulceration.

In this article a further example of intractable diarrhoea (the daily stool volume being accurately known) associated with a non-insulin-secreting pancreatic tumour is recorded, and the literature is reviewed. We suggest either that peptic ulceration is not necessarily part of the Zollinger-Ellison syndrome, which in any case must be enlarged to include diarrhoea and steatorrhoea, or that the association of intractable diarrhoea, hypokalaemia, hyponatraemia, and islet cell tumours of the pancreas (excluding those arising in the insulin-secreting B cells), constitutes a syndrome entirely distinct from that described by Zollinger and Ellison.

CASE REPORT

On 13 March 1958 a married Jewess, aged 55, was referred with a history of loss of weight, thirst, and frequency of 28 days' duration, and of pruritus vulvae for 10 days. Examination revealed only glycosuria, marked anxiety, and loss of weight. A glucose tolerance test on 18 March confirmed the diagnosis of diabetes mellitus, the blood sugar values being 130 mg.% fasting, and 260, 340, 330, and 260 mg.% respectively. Sugar was present in all urines except the fasting specimen; ketone bodies were absent. Early admission was arranged, but the patient defaulted.

Her previous history was of good general health. In 1940 a right simple mastectomy had been carried out for recurrent abscesses (she was childless). In August 1957 she was very thoroughly investigated by Professor L. N. Pyrah for what appeared to be a left pyelitis with frequency, brief haematuria, and some dysuria. Culture of the urine, cystoscopy, and intravenous pyelography were all negative in September: she was given symptomatic treatment, including a course of oxytetracyclin, and her symptoms disappeared. These three examinations were repeated in December 1957, again with negative findings. During the last four months of 1957 she had had recurrent attacks of diarrhoea, with intervals of complete freedom. The motions varied from three to five a day, and were said to be loose and creamy. The diarrhoea seemed to have begun when she was receiving oxytetracyclin, and she received independent treatment for spastic colon.

She was seen again by M.T. on 16 September 1958, this time urgently at her home for gross diarrhoea and loss of weight. It transpired that after she had refused admission for stabilization of her diabetes, diarrhoea returned sharply, and she had seen another consultant who admitted her to hospital. Radiological investigation by barium meal and enema was negative, and the diabetes was said to have been controlled by a Lawrence diet of 12 black and nine red lines. She continued, however, to lose weight, and the diarrhoea soon returned. In June 1958, she sought the advice of a consultant in London, and was in hospital for seven weeks, returning home at the end of August. A barium meal and enema were repeated with negative findings, the diabetes was stabilized by diet and 20 units of Lente insulin daily, and she gained 2 stones in weight. The replacement of Lente insulin by Tolbutamide was followed rapidly by a return of diarrhoea, and she was mildly diarrhoeic at the time of discharge. The alkaline phosphatase was noted to be 67 units. Immediately after her return to Leeds at the end of August, diarrhoea became much worse, and when she was seen at home on 16 September, she was dehydrated, grossly emaciated, and unable to control the almost continuous passage of watery stools into her bed. Her blood pressure was 120/80 mm. Hg, the abdomen flatulent and distended, but examination was otherwise unremarkable. She was admitted to the General Infirmary at Leeds on 17 September.

SUBSEQUENT CLINICAL PROGRESS This may be summed up as a continuous metabolic battle, characterized by slow but relentless clinical deterioration, persistent hypokalaemia and hyponatraemia, ending by death on 16 December in electrolytic chaos, and high-lighted throughout by the passage of fantastically large, featureless, watery fluid stools, the daily totals regularly exceeding 4 and 5 litres, and on three occasions 6 litres, the highest being 6,900 ml. Permission for necropsy was refused.

Clinically the main features were steady decline and loss of weight, frequent vomiting, and inexorable dehydration. She was always afebrile, and her pulse rate ran between 72 and 96. No abnormality was ever found in the abdomen, though it was at times 'doughy'. All other systems were always normal. Repeated blood counts showed only haemoconcentration in the early stages, typical findings being, R.B.C.s 5·4 million and Hb 109%, and in the later, secondary anaemia of the order of Hb 66% and R.B.C.s 3·3 million. Two barium meals and two barium enemas had been reported normal elsewhere; Professor A. S. Johnstone carried out a barium enema examination in September, and barium meals on the 24 September, 22 October, and 11 November 1958. He found no evidence of peptic ulceration in stomach, duodenum, or jejunum, no sign of fistula formation, and no disease of the colon. The colon was observed to function 'like a large conduit', and some flocculation of the barium was seen in the terminal ileum. Three sigmoidoscopies were negative. Stool examination and culture were repeatedly quite unhelpful, though once *Staphylococcus aureus* was grown: treatment with erythromycin was rapidly and permanently effective in as much as the stools became negative for staphylococci on culture, but the general course of the illness was not affected. The total plasma proteins and albumin/globulin ratios were normal on five occasions.

The serum electrolytes and plasma CO_2-combining power were estimated on 38 occasions: values for serum sodium ranged from 113 to 139 mEq./l, for serum potassium 2·2 to 3·8 mEq./l, for serum chloride 78 to 100 mEq./l, and for CO_2-combining power 15·5 to 24 mEq./l. Serum calcium and phosphorus were estimated weekly, values for the former ranging from 9·5 to 13·0 and for the latter 0·8 to 2·1 mEq./l respectively. Red cell potassium on 25 November 1958 and 4 December was 86 and 96 mEq./l. The alkaline phosphatase values at approximately weekly intervals were 45, 47, 52, 20, 7, 21, and 18 units.

Certain other investigations were carried out.

1 *Faecal fat excretion* On 6 October 1958, output, 7%, containing 6% split, 1% neutral, fat; on 14 October, intake 150 g. over 72 hours and output 25 g. a day.

2 *Hydroxyindole acetic acid excretion* On 30 October and 23 November, nil.

3 17-*Ketosteroid excretion* 4·8 mg. in 24 hours.

4 *Urinary calcium excretion on normal ward diet* 310 mg. (volume = 830 ml.).

5 *Urinary potassium excretion* On 27 November, 39 mEq./l.; on 1 December, 7·9 mEq./l.

6 *Urinary chloride excretion* On 27 November, 58 mEq./l.; on 1 December, 21·4 mEq./l.

7 Tests for arsenic, antimony, mercury, copper, lead, zinc, and thallium poisoning were negative.

8 An E.C.G. on 20 November showed changes

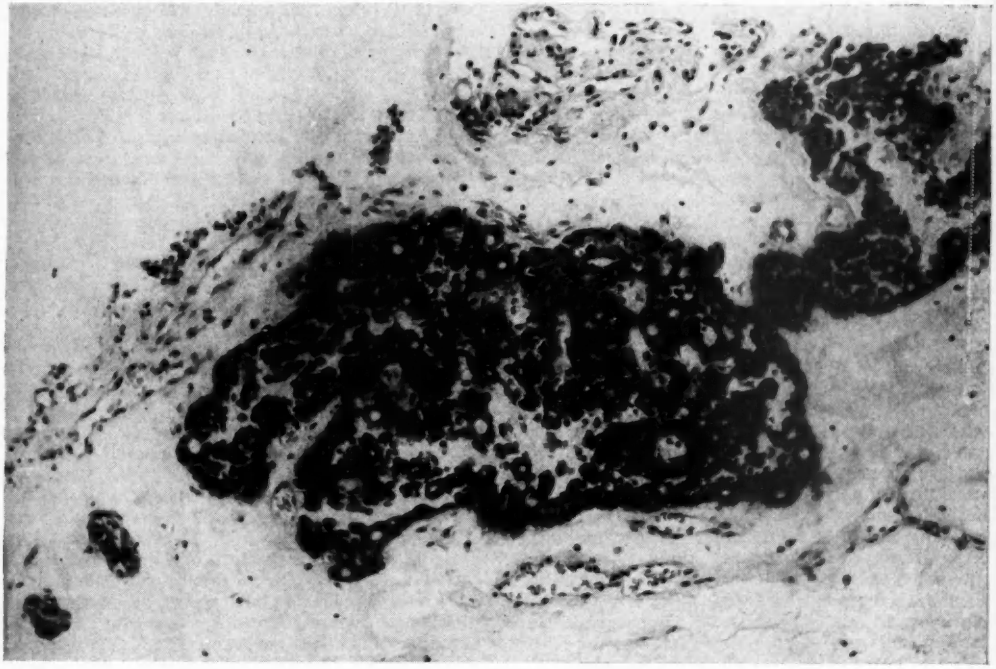

FIG. 1. *Tumour cells forming papillary processes, islets, and strands in a partly hyaline stroma. The tumour cells are deeply stained with regular nuclei; the staining reaction of the intracellular granules suggests that the cells are of alpha type (haematoxylin and eosin* × 300).

consistent with hypokalaemia, which had disappeared on 29 November.

9 On 18 October carmine appeared in the stools six hours after a capsule had been given orally.

TREATMENT The diabetes was always easily controlled by a 12 black and eight red line diet, and some 20 units of Lente insulin daily. It was noted that whenever metabolic control improved the number of daily motions fell, though the total volume remained unchanged.

The persistent loss of fluid, sodium, and potassium was corrected by daily oral or parenteral replacement, regularly reaching a maximum of 5 litres and as much as 6 g. of KCl or $KHCO_3$. Despite this it was only with difficulty that hypokalaemia and dehydration could be avoided. Symptomatic treatment of the diarrhoea with a gluten-free diet, salazopyrine, and antispasmodics such as Probanthine, was completely ineffective. Prednisolone, 10 mg., t.d.s., and later cortisone, 50 mg., b.d. increasing to 50 mg., six hourly, produced no appreciable diminution in the volume of faeces. In the course of her steroid therapy, she had a small haematemesis on the 5th and 11th November, the cause being uncertain. No transfusion was needed.

On 5 December her condition had improved sufficiently to allow Mr. A. J. C. Latchmore to carry out a long-awaited laparotomy. The only abnormality was a large and obviously malignant growth involving the body and tail of the pancreas; the growth was extremely soft and vascular, and inoperable. There was no evidence of peptic ulceration. A biopsy was taken, and Dr. T. W. Sutherland reported as follows:—

'The biopsy material was stained by haematoxylin-eosin, phosphotungsic acid haematoxylin, Masson's trichrome stain, and Gomori's chrome-alum haematoxylin. Microscopically columnar and polyhedral epithelial cells form slender strands intermingled with densely hyaline stroma. In some parts the appearances suggest an adenopapillary pattern. Gomori's stain reveals red granules in most of the cells. The findings indicate that this is an adenocarcinoma (Fig. 1) of low-grade malignancy arising in the alpha or delta cells of the Islets of Langerhans'.

DISCUSSION

Verner and Morrison (1958) noticed particularly the refractory watery diarrhoea in their two patients,

and were able to collect from the literature seven further patients. In five of these the diarrhoea preceded the symptoms of peptic ulcer by many years, a point noted originally by Zollinger and Ellison (1955), later by Donaldson, vom Eigen, and Dwight (1957), Maynard and Point (1958), Summerskill (1959), and Rawson *et al.* (1960). The association of steatorrhoea with the Zollinger-Ellison syndrome has been recorded by Maynard and Point (1958), Summerskill (1959), and Rawson *et al.* (1960). The most acceptable explanation is incomplete neutralization of the large amount of HCl by pancreatic bicarbonate in the small bowel, the low *p*H impairing emulsification and lipolysis.

We suggest that non-insulin-secreting islet cell tumours of the pancreas may be responsible for more than one clinical syndrome. It is clear that profuse intractable watery diarrhoea, hypokalaemia, and hyponatraemia are associated with such pancreatic islet cell tumours, that peptic ulceration may either never develop or that many years may elapse before it finally appears. Table I summarizes 11 case histories, 10 previously recorded and the present one. There seems no doubt that clinicians generally are not fully aware of the association of intractable diarrhoea and islet cell tumours of the pancreas: few examples so far have been recorded.

Only four of the 10 patients in Table I developed peptic ulceration, and in each it had been preceded by a long period of diarrhoea; in one it was terminal. This is very different from the Zollinger-Ellison syndrome, as generally defined, in which peptic ulceration and gastric hypersecretion appear early and are the dominant features. The absence of peptic ulceration in seven, and its late appearance in four, of the patients in Table I, certainly suggest that excessive acid secretion was not the prime cause in these 11 patients. It is true that in only a few case reports has the degree of gastric hypersecretion been measured (Zollinger and Ellison, 1955; Donaldson *et al.*, 1957; Maynard and Point, 1958; Summerskill, 1959; Rawson *et al.*, 1960). A further difficulty is the absence of precise analysis of the faecal discharge; we have been able to discover no report of the electrolytic composition of the faeces, and only in one patient (Rawson *et al.*, 1960) has the faecal volume been measured. On the other hand removal of the tumour in one patient (Brown, Neville, and Hazard, 1950) relieved the diarrhoea. Improvement occurred in Pender's (1959) patient after removal of a pancreatic islet cell tumour, but the most successful result is that described by Rawson *et al.* (1960) in whose patient removal of the tumour produced complete relief of symptoms and reduced the excessive secretion of acid by the stomach.

The mechanism of the severe faecal loss, which in our patient varied from 1,750 to 6,900 ml. daily, must clearly be caused in one of two ways; failure of the absorptive mechanism of the gastrointestinal tract, or increased output of fluid and electrolytes by the gastrointestinal tract. Since a terminal ileostomy normally discharges less than 1 litre of fluid daily which is isotonic with plasma (Smiddy, Smith, Gregory, and Goligher, 1960), it appears most likely that the mechanism involved is oversecretion and hurry. This hypothesis is substantiated by two further facts: first, in five of the 11 patients fat balance studies were made; one had steatorrhoea, but in four normal or near-normal values of faecal fat excretion were found, thus suggesting that the absorptive capacity of the small bowel was essentially normal; secondly, though cortisone normally lowers the fluid output of the small bowel as a result of increased absorption of water and sodium (Smiddy *et al.*, 1960), in our patient 200 mg. of cortisone daily produced no diminution in the faecal loss. The possibility also arose that this patient was suffering from hypercatabolic hypoproteinaemia, in which condition diarrhoea has occasionally been reported (Schwartz and Jarnum, 1959), but oedema was never observed and the serum protein levels remained within normal limits throughout.

It is unfortunate that the histological studies of all the tumours so far reported are of little help, because the methods used failed to identify the various islet cell types (Gomori, 1941). Even in the patient described by Rawson *et al.* (1960) the results were equivocal despite the most modern techniques. The most that can be said from all the reports, including our own, is that the islet cell tumours are not of the insulin-secreting B cell type. Our patient appears to be alone in having proven diabetes in addition.

Solitary tumours were present in nine, and multiple adenomata in two, patients (Table I); five were benign, four malignant, and in two patients the nature of the tissue is not stated. Lymph node metastases were present in one. The proportion of malignant tumours in these 11 patients is much lower than Ellison's (1956) series of 24, of which 19 were malignant. The rate of growth varies considerably; the tumours may remain small, and in three patients were not found at laparotomy. Where excision has been successful, the clinical results appear so far to be good (Brown *et al.*, 1950; Rawson *et al.*, 1960).

The clinical picture of the 11 patients set out in Table I differs significantly from that described by Zollinger and Ellison in four main respects; profuse intractable watery diarrhoea is present for several years; hypokalaemia develops concurrently, and is the commonest mode of death (six of 11 patients);

TABLE I

CLINICAL SUMMARY OF 11 CASES

	Author	Sex	Age	Presenting Symptoms	Duration of Bowel Symptoms	Previous Operation	Hypokalaemia	Peptic Ulceration	Fat Absorption	Laparotomy	Cause of Death	Necropsy	Cell Type	Nature of Growth
1	Zollinger and Ellison (1955)	F	36	Diarrhoea, loss of weight	8 yr.	No	Suspected on clinical history given	Developed after 5 yr.	Not investigated	Jejunal ulcer	Duodeno-oesophago-cutaneous fistula	Islet cell tumour (small)	Unknown	Benign
2	Moldawer *et al.* (1954)	F	57	Diarrhoea, loss of weight	4 yr.	No	Renal tubular changes found at necropsy suggested long-standing hypokalaemia	Absent	Normal	Not done	Hypokalaemia	Islet cell tumour (large)	Unknown	Benign
3	Priest and Alexander (1957)	F	56	Diarrhoea, loss of weight	6 mth.	Excision of pancreatic neoplasm 5 yr. before development of diarrhoea	Proved	Absent but gastric ulcer developed during steroid therapy	Normal	Negative	Hypokalaemia	Islet cell tumours (small)	Unknown	Not stated probably malignant
4	Donaldson *et al.* (1957)	M	35	Diarrhoea, tetany, hypochloraemic alkalosis	2 yr.	No	Proved	Perforated jejunal ulcer	Normal	Perforated jejunal ulcer with peritonitis	Hypokalaemia + peritonitis	4 peptic ulcers, 2 islet cell tumours	Unknown	Malignant
5	Forty and Barrett (1952)	F	55	Diarrhoea, loss of weight, recurrent epigastric pain	3 yr.	No	Not investigated	Associated ulcer 3rd part of duodenum	Not investigated	Duodenal ulcer 3rd part	Perforation of recurrent ulcer post-operatively	2 islet cell tumours, ulceration of jejunum opposite gastrojejunal stoma	Unknown	Unknown
6	Brown *et al.* (1950)	F	74	Diarrhoea, loss of weight	4 mth.	No	Not investigated	Absent	Not investigated	Tumour of pancreas	Survived; after resection of pancreas, diarrhoea subsided	Islet cell tumour (large)	α-cell	Benign
7	Verner and Morrison (1958)	M	67	Diarrhoea, loss of weight	10 mth.	No	Proved	Absent	Normal	Not done	Hypokalaemia	Medium islet cell tumour, chromophobe adenoma of pituitary	Unknown	Benign
8	Verner and Morrison (1958)	M	19	Diarrhoea, loss of weight	3 yr.	Exploration of parathyroids	Proved	Absent	Normal	Not done	Hypokalaemia	Islet cell tumour (? size)	Not β cell	Benign
9	Gordon and Olivetti (1947)	M	26	Diarrhoea, loss of weight, acute onset of upper abdominal pain	1 yr.	No	Not proven, complained of progressive weakness	Perforated oesophageal ulcer after 1 yr. associated with 3 chronic duodenal ulcers	Not investigated	Jejunostomy only	Perforated oesophageal ulcer	Multiple pancreatic tumour and adrenal cortical adenomata	Unknown	Malignant
10	Smiddy and Telling (1961)	F	47	Diarrhoea, loss of weight	13 mth.	No	Proved	No ulcer at operation, haematemesis during steroid therapy	Slight	Tumour of pancreas	Hypokalaemia	Not done	δ-cells	Malignant
11	Maynard and Point (1958)	M	53	Diarrhoea, loss of weight, abdominal pain	4 yr.	None for relevant condition, appendicectomy, cholecystectomy	Not proved, 'non-tropical sprue' diagnosed	Absent'	Gross, diagnosed as 'non-tropical sprue'	Large tumour of pancreas, no ulceration found at 2 laparotomies	Malignant cachexia and vomiting	Multiple terminal jejunal ulcers (one had perforated)	Carcinoma of islets of Langerhans	Malignant

the absence or very late appearance of peptic ulceration; and a lower malignancy rate. Steatorrhoea seems to be associated more with the Zollinger-Ellison syndrome, but more information is needed before its importance can be accurately assessed.

This leads to the attractive hypothesis that there may be two different types of non-insulin-secreting islet cell tumour of the pancreas: the one may cause gastric oversecretion and peptic ulceration; the other intestinal oversecretion, diarrhoea, and hypokalaemia. How these different reactions are mediated unfortunately remains quite obscure. It is tempting to postulate a hormonal mechanism, but better understanding will only come from further studies of patients with chronic severe diarrhoea or obstinate peptic ulceration, made with particular regard to the possible presence of islet cell tumours.

SUMMARY

In this paper we have stressed the association between severe watery diarrhoea and islet cell tumours of the pancreas. The clinical picture of this syndrome appears to differ sharply from that of the classical Zollinger and Ellison syndrome in that the clinical course of the disease is prolonged, is dominated by diarrhoea with an attendant severe hypokalaemia, and there is no or very late peptic ulceration. In addition, in the majority of patients there is no steatorrhoea and a lower incidence of malignancy. Comparison, therefore, of these two syndromes suggests that there may be two different types of non-insulin-secreting islet cell tumours but this hypothesis cannot be substantiated in the absence of clear histological methods of differentiating between islet cell types.

We are indebted to Dr. F. M. Parsons, Assistant Director of the M.R.C. Unit, for his help in the control of the electrolyte disturbances, and to the nursing staff for their unfailing care and skill.

REFERENCES

Brown, C. H., Neville, W. E., and Hazard, J. B. (1950). Islet-cell adenoma, without hypoglycemia, causing duodenal obstruction. *Surgery*, **27**, 616-20.

Donaldson, R. M., vom Eigen, P. R., and Dwight, R. W. (1957). Gastric hypersecretion, peptic ulceration and islet-cell tumor of the pancreas (the Zollinger-Ellison syndrome); report of a case and a review of the literature. *New Engl. J. Med.*, **257**, 965-70.

Ellison, E. H. (1956). The ulcerogenic tumor of the pancreas. *Surgery*, **40**, 147-170.

Forty, F., and Barrett, G. M. (1952). Peptic ulceration of the third part of the duodenum, associated with islet-cell tumours of the pancreas. *Brit. J. Surg.*, **40**, 60-63.

Gomori, G. (1941). Observations with differential stains on human islets of Langerhans. *Amer. J. Path.*, **17**, 395-406.

Gordon, B. S., and Olivetti, R. G. (1947). Carcinoma of the islets of Langerhans; review of the literature and report of two cases. *Gastroenterology*, **9**, 409-424.

Maynard, E. P., and Point, W. W. (1958). Steatorrhea associated with ulcerogenic tumor of the pancreas. *Amer. J. Med.*, **25**, 456-459.

Moldawer, P. M., Nardi, G. L., and Raker, J. W. (1954). Concomitance of multiple adenomas of the parathyroids and pancreatic islets with tumor of the pituitary: a syndrome with familial incidence. *Amer. J. med. Sci.*, **228**, 190-206.

Pender, B. (1959). Islet-cell tumour of pancreas, associated with peptic ulceration. *Lancet*, **1**, 123-124.

Priest, W. M., and Alexander, M. K. (1957). Islet-cell tumour of the pancreas with peptic ulceration, diarrhoea and hypokalaemia. *Ibid*, **2**, 1145-1147.

Rawson, A. B., England, M. T., Gillam, G. G., French, J. M., and Stammers, F. A. R. (1960). Zollinger-Ellison syndrome with diarrhoea and malabsorption. *Ibid*, **2**, 131-134.

Schwartz, M., and Jarnum, S. (1959). Gastrointestinal protein loss in idiopathic (hypercatabolic) hypoproteinaemia. *Ibid*, **1**, 327-330.

Smiddy, F. G., Smith I. B., Gregory, S. D., and Goligher, J. C. (1960). Faecal loss of fluid, electrolytes, and nitrogen in colitis before and after ileostomy. *Ibid*, **1**, 14-19.

Summerskill, W. H. J. (1959). Malabsorption and jejunal ulceration due to gastric hypersecretion with pancreatic islet-cell hyperplasia. *Ibid*, **1**, 120-123.

Verner, J. V., and Morrison, A. B. (1958). Islet-cell tumor and a syndrome of refractory watery diarrhea and hypokalemia. *Amer. J. Med.*, **25**, 374-380.

Zollinger, R. M., and Elliott, D. W. (1959). Pancreatic endocrine function and peptic ulceration. *Gastroenterology*, **37**, 401-411.

——, and Ellison, E. H. (1955). Primary peptic ulcerations of the jejunum associated with islet-cell tumors of the pancreas. *Ann. Surg.*, **142**, 709-728.

Gut, 1961, **2**, 18

A study of malabsorption after resection of the entire jejunum and the proximal half of the ileum

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SYNOPSIS Biochemical, haematological, and radiological investigations are reported in a young child in whom nearly 80% of the small intestine was resected. The prognosis in a child after such extensive resection is probably better than in an adult, as natural growth can still occur with the expectation of greater adaptability as well as of increased length of the remaining segments.

The supreme role of the small intestine in digestion and absorption makes it an indispensable organ. Elaboration of the functions of its several parts is of considerable importance because of its involvement at different levels by a variety of diseases and anatomical lesions. Although special techniques (Borgström, Dahlqvist, Lundh, and Sjövall, 1957; Blomstrand, 1958; Cox, Meynell, Cooke, and Gaddie, 1958; Booth and Mollin, 1959) are now giving precision to the study of the physiology of digestion and absorption, it is by no means clear what proportion of normal absorption takes place in the ileum nor what its functional reserve is. For this reason observations on a unique patient who retains only the distal 22% of small intestine are recorded.

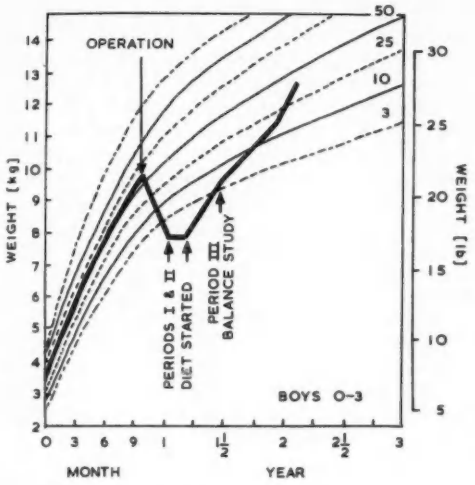
CASE REPORT

C.L., a boy, was born on 20 July 1958, whose birth weight was 3·4 kg. (7½ lb.). Progress and weight gains to 9·72 kg. (21 lb. 6½ oz.) at 10 months were normal. At this age operations for intussusception complicated by gangrene resulted in resection of the small gut from the duodenal-jejunal flexure to past the midpoint in the ileum. At the operations it was estimated that 134·5 cm. (78%) was removed leaving 38 cm. (15 in.) distally. Continuity was restored by gastro-ileostomy and the duodenal stump was closed. He made a good recovery after this remarkable surgery. Initially he had 10 to 14 loose motions daily which settled to seven to nine slightly formed ones at the age of 1 year by which time he had lost 1·36 kg. (3 lb.) in weight (Fig. 1). He was at that time a bright, underweight child with a protuberant abdomen. His diet was similar to that of a 6-month-old infant, and it was noted that milk feeds tended to pass through quickly. He was subject to attacks of prostration with episodes of vomiting and diarrhoea.

It was at that stage, two months post-operatively, that our investigations began. His haemoglobin was 88% (12·8 g.), red and white blood cell counts were normal, reticulocytes 0·6%, and prothrombin time normal. Serum B_{12} was 285 and 310 $\mu\mu$g./ml. and B_{12} absorption was 0·41 μg. after a dose of 1 μg. of $Co^{58}B_{12}$. Biochemical examination of capillary blood (Wilkinson, 1960) gave the following results: total plasma protein 6·3 g./100 ml. with a normal electrophoretic pattern apart from a moderate increase in gamma globulin, serum bilirubin 0·5 mg./100 ml., and plasma values for calcium 9·4 mg./100 ml., phosphorus 4·4 mg./100 ml., alkaline phosphatase 17 K.A. units/100 ml., bicarbonate 20 mEq./l., chloride 107 mEq./l., sodium 137 mEq/l., potassium 4·2 mEq./l., blood urea 47 mg./100 ml. The fasting blood sugar was 92 mg./100 ml. and at half-hourly intervals after 13 g. glucose (Poulton-Payne dosage: Wilkinson, 1960) was 118, 100, 78, 89, and 78 mg./100 ml.

A barium meal examination showed normal gastric emptying in the course of one and three-quarter hours by which time a little barium had reached the caecum. The ileum was still well filled at three hours and was clear at four hours. The ileum was considerably dilated except in the last few inches.

The first balance study (periods I and II) was carried out using a diet which closely resembled the one he had been having up to that time. The diet contained 980 calories, half of them derived from fat and the main constituents of it were baby foods and milk (2 pints). Although he had been losing weight steadily on this kind of food there was no change in his weight of 7·9 kg. during the balance period. Nevertheless, since his ideal weight for age would have been 10·3 kg. (Fig. 1) this diet was inadequate calorifically even if it had been totally assimilated. In order to recover the weight lost, which now amounted to 1·82 kg. (4 lb) or nearly one fifth of



Patient's Age (mth.)	*Weight (lb. oz.)*	*Other*
At birth	7½	
10	21 6½	Operation
12	18 6	
13	17 6	First balance study
14	17 6	Diet initiated
15	18 6	
15½	19	
18	21 4	
19	22 4	Second balance study
20½	23 6	
22	24 5	
23	25 4	
25	27 4	

FIG. 1. *Standard weight chart (Tanner, 1958)*

what his weight had been at 10 months, his requirements were very much greater than those of a normal child.

Accordingly a diet high in protein and calories with low fat and roughage content in four main meals daily was instituted. Milk, because of its observed deleterious effects, was limited to 1½ pints daily and proteins were given in solid form (chopped meats). Improvement was immediate on this régime both in weight and wellbeing. Bowel motions were reduced to three formed motions and he quickly recovered his admission weight of 8·45 kg. before discharge. Subsequently, after his return home weight gains averaged 0·34 kg. (12 oz.) a month (Fig. 1) and he became an active and energetic boy.

He continued to do well except for an interlude at the time of the second balance study (period III) six months later. At that time less care was being exercised in regard to the fat and milk content of his diet. When these were again reduced, to the extent of avoiding all obvious fat and limiting the milk to a daily total not exceeding 1½ pints to be taken between rather than with meals, the stools returned to two daily of moderate size and the steady weight gains continued as before (Fig. 1). Investigations repeated after the second balance, that is nine months after the operations, were as follows: total plasma protein 6·7 g./100 ml.; plasma calcium 9·5 mg./100 ml., plasma phosphorus 5·0 mg./100 ml., plasma alkaline phosphatase 15 K.A. units/100 ml., serum B_{12} 445 $\mu\mu$g./ml. Cell counts and blood film appearances were normal.

THE BALANCE STUDIES

Periods I and II were two months after the resection and period III was six months later, the patient's age being 12 and 18 months respectively.

The first balance study was carried out in hospital, the same diet being given each day. Food and fluids were prepared in double quantity by the hospital diet kitchen, half being sent to the laboratory for analysis and half being given to the child. All food and fluids refused by the patient were sent to the laboratory for analysis. Food was reduced to a uniform paste with a mechanical blender for analysis at the end of each metabolic period. Precautions were taken to exclude extraneous sources of calcium, and distilled water was used throughout. Carmine powder, 3 grains by mouth, was used as a stool marker at the beginning and end of each metabolic period. Stools were collected separately into parchment containers and bulked at the end of each period. Twenty-four-hour collections of urine were bulked at the end of each period. No urine or stool was lost.

The second balance study (period III) was performed at home with the cooperation of the mother. It was carried out in the same way as the first study, except that kitchen scales were used to weigh the food, the diet varied from day to day, and tap water was used (but an equal amount was analysed). During this study some urine was lost by bedwetting at night, but since the mother potted the child hourly she was able to assure us that the amount lost was very small. His weight during this study was 10·3 kg.

ANALYTICAL METHODS Wet ashing of samples of food, faeces, and urine was employed, using sulphuric acid and hydrogen peroxide for calcium and phosphorus, sulphuric acid for nitrogen, and nitric acid for sodium and potassium.

Nitrogen in food, faeces, and urine was determined by the standard micro-Kjeldahl procedure, phosphorus by the method of Briggs (1922), calcium by the method of Tisdall and Kramer (1921), and sodium and potassium by a single-cell, direct-reading flame photometer. Fat in food and faeces was determined by a slight modification of the wet extraction method of van de Kamer, Huinink, ten Bokkel, and Weyjers (1949).

The results of the metabolic studies are shown in Table I.

DISCUSSION

The wide-ranging and variable effects of extensive resections of small intestine have been discussed in many excellent studies and reviews in the last decade (Althausen, Doig, Uyeyama, and Weiden, 1950; Jackson and Linder, 1951, 1955; Berman, Habegger,

TABLE 1

RESULTS OF METABOLIC STUDIES DURING THREE PERIODS

	Period I[1]	*Period II*	*Period III*
Nitrogen			
Intake	22·6	22·6	52·4
Urinary excretion	9·0	7·9	27·0
Faecal excretion	4·3	4·5	11·5
Total excretion	13·3	12·4	38·5
Balance	+9·3	+10·2	+13·9
Mean daily balance	+2·3	+2·6	+2·8
Phosphorus			
Intake	4·2	4·2	6·4
Urinary excretion	0·4	0·5	1·9
Faecal excretion	1·0	1·1	2·5
Total excretion	1·4	1·6	4·4
Balance	+2·8	+2·6	+2·0
Mean daily balance	+0·7	+0·7	+0·4
Calcium			
Intake	3·8	3·8	5·4
Urinary excretion	0·02	0·03	0·15
Faecal excretion	1·7	1·2	3·5
Total excretion	1·72	1·23	3·65
Balance	+2·1	+2·6	+1·8
Mean daily balance	+0·5	+0·6	+0·4
Fat			
Intake	194·0	194·0	158·8
Faecal excretion	48·4	53·2	128·0
Balance	+145·6	+140·8	+30·8
Percentage absorption	75	72	19
Sodium			
Intake	91	91	353
Urinary excretion	29	48	232
Faecal excretion	11	12	25
Total excretion	40	60	257
Balance	+51	+31	+96
Mean daily balance	+13	+8	+19
Potassium			
Intake	203	203	256
Urinary excretion	30	15	139
Faecal excretion	52	50	72
Total excretion	82	65	211
Balance	+121	+138	+45
Mean daily balance	+31	+34	+9

[1]Periods I and II were four days and period III was five days. Results for nitrogen, phosphorus, calcium, and fat are given in grams, and for sodium and potassium in milliequivalents.

and Billings, 1953; Pilling and Cresson, 1957; Pullan, 1959). Early reports were more concerned with the extent of the resection whereas the emphasis is now on the nature and function of remaining segments. The present patient is exceptional in having no jejunum at all, and a duodenum which is now an appendage to the stomach.

Radiological examination three months after his operations showed considerable dilatation of the ileum, a finding which is usually reported as confined to the jejunum in those patients who retain a segment of the latter. Intestinal transit times were not unduly increased, but because there are many factors which influence the passage of barium as well as a wide normal variation, the exact significance of these times is elusive. However, this examination did suggest that possession of the ileo-caecal valve and of terminal ileum was controlling the rate of passage through the remaining small gut.

Because detailed studies have rarely been done on children when the demands for growth and nutrition are proportionately so much greater there was a special interest in our study of this patient. It was noteworthy that he made such a good post-operative recovery after a quick succession of operations at the age of 10 months when he was extremely ill with gangrene and perforations. Although losing weight rapidly at first, he was able to maintain his bodily equilibrium to the extent that two months later all haematological and biochemical investigations were within the range of normal, apart from evidence of mild dehydration.

As expected, since the ileum is the main site of B_{12} absorption (Booth and Mollin, 1959), the serum B_{12} levels and $Co^{58}B_{12}$ uptake were normal (Mollin, Booth, and Baker, 1957). The glucose tolerance figures were within the range of normal in our experience with the method used and the stool fat at that time was 12-13 g. daily. This combination of normal B_{12} and glucose absorption with high stool fat gives an additional pattern to three types described by Booth (1960) in association with distal resections.

The balance study of two consecutive periods (I and II) shows great uniformity at a time of relative stability for the patient in respect of previous diet and constant weight at the time of the study. Although the diet was unsuited to his needs, due to high fat and milk content, he retained 75% of 48·5 g. of fat. A nitrogen intake of 5·66 g. was less than desired in his case, but approximately normal for his 'ideal' weight of 10·3 kg. equivalent to 3·5 g. of protein/kg. (League of Nations Report on the Physiological Basis of Nutrition, 1936). The study showed a positive balance of 40 to 45% equivalent to 1·5 g. of protein/kg. daily and normal urinary and faecal excretion of nitrogen. There was an excellent positive balance for calcium, phosphorus, sodium, potassium, and chloride in spite of the quite inadequate sodium chloride intake. From these results it was clear that he could absorb adequate protein, minerals, and electrolytes as well as a moderate amount of fat. Although we did not know of any comparable study the malabsorption was less severe than expected from reports in the literature, and we attributed this to the efficiency of absorption in the ileal segment.

Six months later we expected increased efficiency of absorption to account for his striking clinical recovery (Fig. 1 and case report), but period III coincided with a short episode of increased steatorrhoea. This event was partly due to laxity about fat, but more particularly to the excessive milk

allowance in an otherwise adequate diet. The ritual of frequent 'potting' to ensure the collection of all excreta undoubtedly promoted intestinal hurry since the stools returned to normal almost immediately afterwards. Although this second study represents an isolated incident certain valid inferences can be drawn from it. In particular it supports the view that there is a high correlation between severity of the steatorrhoea and degree of malabsorption of the other dietary constituents in such cases (Pietz, 1956). On this occasion it mainly affected the absorption of calcium and phosphorus, since 78% of the protein intake in period III was absorbed compared with 80% in the first study. The study confirmed the clinical observation of the deleterious effect of an excessive intake of milk because the intake of fat during period III was only 66% of that pertaining to periods I and II, whereas the child had been given a greatly increased amount of milk.

In period III the stool fat had more than doubled to 25·6 g. a day so that only 19% of 32 g. of dietary fat was retained. At the same time an abnormal amount of nitrogen was lost in the stools (2·3 g. or 22% of intake) in association with the increased intake of 10·5 g. However, the percentage of protein absorbed (78%) was very satisfactory, and although there was a positive balance of only 26% due to the greatly increased urinary nitrogen the net nitrogen retention was maintained at a level equivalent to 1·5 g. of protein/kg. of his 'ideal' weight (11·7 kg.) for age at 18 months. Calcium and phosphorus lost in the stools was more than doubled but the total absorbed was similar to that absorbed in periods I and II. The percentages of sodium and potassium taken up were also greatly reduced, but the more normal urinary outputs, together with increased total intake, suggests a satisfactory equilibrium. The reduced percentages of intake absorbed in period III suggested exceptional intestinal hurry due to the unusual conditions of the study and cannot be regarded as representative of his 'normal'. These findings show the vulnerability of such a patient to injudicious changes of diet and routine as well as the very worth while measure of function achieved by a small segment in such adverse circumstances. Since period III, with stricter adherence to diet, he has continued his steady weight gains (Fig. 1), and stools have returned to his usual one to two formed motions of normal size.

The diet which produced such an excellent clinical response and control of his motions without any additional measures whatever is essentially similar to the recommendations of the authors quoted. The special features which we wish to emphasize are limitation of milk intake and avoidance of liquid protein concentrates. The natural tendency of parents, nurses, and dietitians to revert to milk and an infant type diet in the belief that food of that kind is more easily assimilated must be resisted. A child of 1 year is quite capable of taking a more solid adult type diet provided it meets his nutritional requirements and that meat, etc., is suitably cut up. Our régime consisted of four main protein-containing meals a day, with snacks and 4 oz. milk drinks from the ration between rather than with meals. The widest possible choice of foods is encouraged, eliminating only the well-known sources of fat and roughage. This has proved both a manageable and expensive diet (but much less costly than various alternatives), and seems to be well suited to the patient's requirements.

Development and attainments have not been retarded and his striking weight recovery in 12 months brought him near to the 50th percentile again at the age of 25 months (Fig. 1). Because the length of the gut is said to increase at least twofold with body growth (Pilling and Cresson, 1957), further improvement and an increasing margin of safety in small intestinal function can be anticipated.

SUMMARY AND CONCLUSIONS

Biochemical, haematological, and radiological investigations are reported, together with detailed metabolic studies on a child two and eight months after resection of 78% of the small intestine. The findings are considered in the light of an anatomical situation where the distal half of the ileum is required to subserve all the remaining functions of the mesenteric small intestine.

The distal half of the ileum appears to be capable of undertaking a substantial amount of the function of the small gut. In this patient B_{12} and glucose were normally absorbed, associated with a raised excretion of fat, giving a distinct pattern of malabsorption. His absorption of protein, minerals, and electrolytes was adequate.

Dilatation and adaptation of the ileum to subserve a role similar to more proximal segments appears to occur in man in a manner similar to the jejunal dilatation reported after distal resections. In addition retention of the terminal ileum and ileocaecal valve is probably especially advantageous after extensive resections in controlling transit times and thereby promoting more efficient absorption.

The impaired absorption of the dietary constituents involved in the malabsorption appears to correlate more closely with the severity of steatorrhoea than with the fat intake when the total absorbing surface of the small intestine is reduced by resection.

Excellent weight gains, together with effective

control of the motions, were achieved with diet alone. The essentials of diet are high protein and calorie, and low fat and roughage content. The need to give protein in solid form and to limit the milk intake has been stressed. Four main meals daily were found to be well suited to this patient's requirements.

The prognosis in a child after such an extensive resection is probably better than in the adult, because natural growth is still occurring, with the expectation of greater adaptability as well as of increased length of the remaining segments.

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REFERENCES

Althausen, T. L., Doig, R. K., Uyeyama, K., and Weiden, S. (1950). *Gastroenterology*, **16**, 126-139.

Berman, J. K., Habegger, E. D., and Billings, E. (1953). *Amer. J. dig. Dis.*, **20**, 152-156.

Blomstrand, R. (1958). Abstracts of Sci. Proc. World Congress of Gastroenterology, Washington, 1958, Vol. I, pp. 644-648. (publ. 1959), by Williams and Wilkins, Baltimore.

Booth, C. C. (1960). *Brit. J. Radiol.*, **33**, 201-211.

——, and Mollin, D. L. (1959). *Lancet*, **1**, 18-21.

Borgström, B., Dahlqvist, A., Lundh, G., and Sjövall, J. (1957). *J. clin. Invest.*, **36**, 1521-1536.

Briggs, A. P. (1922). *J. biol. Chem.*, **53**, 13-16.

Cox, E. V., Meynell, M. J., Cooke, W. T., and Gaddie, R. (1958). *Gastroenterology*, **35**, 390-397.

Jackson, W. P. U., and Linder, G. C. (1951). *S. Afr. J. clin. Sci.*, **2**, 70-112.

——,—— (1955). *Acta med. scand., Suppl.*, **306**, 96-106.

Kamer, J. H. van de, Huinink, H. ten Bokkel, and Weyjers, H. A. (1949). *Ibid*, **177**, 347-355.

League of Nations Report on Physiological Basis of Nutrition (1936). Technical commission appointed by the Health Committee, London, 1936. H.M.S.O., London.

Mollin, D. L., Booth, C. C., and Baker, S. J. (1957). *Brit. J. Haemat.*, **3**, 412-428.

Pietz., D. G. (1956). *Gastroenterology*, **31**, 56-73.

Pilling, G. P., and Cresson, S. L. (1957). *Pediatrics*, **19**, 940-948.

Pullan, J. M. (1959). *Proc. roy. Soc. Med.*, **52**, 31-37.

Tanner, J. M. (1958). In *Modern Trends in Paediatrics* (*2nd series*), ed. A. Holzel and J. P. M. Tizard, pp. 325-344. Butterworths, London.

Tisdall, F. F., and Kramer, B. (1921). *J. biol. Chem.*, **48**, 1-12.

Wilkinson, R. H. (1960). *Chemical Micromethods in Clinical Medicine*. Thomas, Springfield, Illinois.

Gut, 1961, **2**, 23

Studies on the site of fat absorption

1. The sites of absorption of increasing doses of ^{131}I-labelled triolein in the rat

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SYNOPSIS These studies on fat absorption in rats show that a small dose was relatively rapidly absorbed in the jejunum. With larger doses it was also absorbed in the ileum. When larger doses were given the jejunum responded to the increased load with an increased absorption, and fat was also absorbed in the ileum.

Absorption of different substances does not occur uniformly in the small intestine. Glucose, for instance, is absorbed rapidly from the jejunum but more slowly in the distal small intestine (Cummins and Jussila, 1955; Verzár and McDougall, 1936) and under physiological conditions vitamin B_{12} is predominantly absorbed from the ileum (Booth, 1958; Booth and Mollin, 1959).

Although the site of fat absorption has been studied by several workers, the relative importance of the proximal and distal small intestine in the absorption of fat is uncertain. Kremen, Linner, and Nelson (1954), from observations in dogs subjected to intestinal resection, have concluded that fat is primarily absorbed in the ileum, and the upper ileum is said to play a major rôle in the absorption of ^{131}I-labelled olive oil in the rat (Benson, Chandler, Vansteenhuyse, and Gagnon, 1956). On the other hand, Turner (1958) considers that the jejunum is the main site of absorption of fat in the dog, and Borgström, Dahlqvist, Lundh, and Sjövall (1957) have suggested that in man fat is absorbed in the proximal 100 cm. of the jejunum.

In view of these conflicting reports, we have made a further study of the site of fat absorption in the rat, using ^{131}I-labelled triolein. Animals were killed at frequent intervals during absorption of different doses of labelled fat and the amount of radioactivity in the various parts of the small intestine was measured. The use of ^{131}I-labelled triolein provides a simple method for this purpose. Digestion of the labelled material does not release significant amounts of free iodide (Balint, Pendower, and Ramsey, 1960) and ^{131}I therefore remains bound to triolein during absorption at least until it has reached the intestinal mucosal cell. Studies of thoracic duct lymph after oral administration of ^{131}I-labelled triolein suggest that it may be partially de-iodinated during absorption (Van Handel and Zilversmit 1958), but this probably occurs within the intestinal mucosal cells since free iodide may be released from ^{131}I-labelled triolein by incubation with freshly prepared extracts of intestinal mucosa (Balint *et al.*, 1960).

This paper is concerned with the detection of labelled triolein only during its passage along the intestinal tract and entry into the mucosal cell and the measurement of the total radioactivity in the various parts of the gut is therefore a satisfactory method of tracing the absorption of ^{131}I-labelled triolein. The techniques used were similar to those reported by Parkins, Dimitriadou, and Booth (1960).

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS Laboratory-bred male or female white rats weighing approximately 200 g. were used throughout the experiments.

^{131}I-LABELLED TRIOLEIN Triolein was iodinated with ^{131}I using a modification of the method described by Lubran and Pearson (1958). The ^{131}I-labelled triolein was purified first by dialysis and then by passage through an ion-exchange resin to remove free iodide. The resulting material usually contained a concentration of 30 to 40 mg. of triolein per ml., of specific activity 0·8 to 0·9 μc. per mg. It was tested for purity by ascension chromatography in a butanol/acetic acid/water medium, the distribution of radioactivity in the chromatograms being determined by autoradiography. Chromatograms were also made after incubation of the ^{131}I-labelled triolein with pan-

creatic extracts or with rat intestinal juice for 24 hours. As reported by Baiint and his colleagues (1960), digestion failed to release significant amounts of ^{131}I from the material.

Test doses of 10, 50, 100, and 500 mg. of ^{131}I-labelled triolein in 1-ml. volume were prepared by dilution and the addition of suitable amounts of unlabelled triolein, the radioactivity of the test doses being adjusted to approximately 2 μc. of ^{131}I.

EXPERIMENTAL PROCEDURE

ABSORPTION OF ^{131}I-LABELLED TRIOLEIN BY INTACT ANIMALS Successive doses of 10, 50, 100, and 500 mg. of ^{131}I-labelled triolein were given to a group of six rats. The animals were starved for 12 hours beforehand and the labelled fat was fed from a graduated syringe through a fine polythene tube passed into the stomach. Each animal was then placed in an individual metabolism cage and the faeces were collected for 48 hours, urine being collected separately. The excretion of radioactivity in the faeces of these animals was measured and absorption was calculated by assuming that the radioactive material not recovered in the faeces had been absorbed.

Measurement of radioactivity The faecal specimens were collected in waxed cartons and counted by positioning each carton between two scintillation counters, according to the method described by Lewis and Porter (1960). This counting arrangement made it possible to count the radioactivity without homogenization. The sensitivity of the counter was approximately 60,000 counts per minute per μc of ^{131}I. Background counting rates ranged from 400 to 600 per minute.

DISTRIBUTION OF RADIOACTIVITY IN GASTROINTESTINAL TRACT DURING ABSORPTION Four groups of 21 animals were used. The animals were starved for 12 hours and each group of rats received intragastric test doses of either 10, 50, 100, or 500 mg. of ^{131}I-labelled triolein. Three animals from each group were then killed at each of the following times after the test doses were given: quarter, half, one, one and a half, two, three, and four hours. The gastro-oesophageal junction, pylorus, and rectum were clamped and the entire gastrointestinal tract was removed. The small intestine was divided into four segments of equal length as follows: (1) duodenum and upper jejunum, (2) lower jejunum, (3) upper ileum, (4) lower ileum. The stomach and different segments of the small intestine and colon were then placed in separate containers and dissolved in 20 ml. of a solution containing equal quantities of 40% sodium hydroxide and absolute alcohol, the mixture being incubated overnight at 56°C.

Measurement of radioactivity The radioactivity of the stomach and intestinal specimens was measured in a coronet-type counter similar to that described by Veall and Baptista (1954). With this counter, 1 μc of ^{131}I recorded approximately 9,000 counts per minute. Background counting rates on this counter ranged from 25 to 45 per minute.

RESULTS

ABSORPTION OF TEST DOSES OF ^{131}I-LABELLED TRIOLEIN BY INTACT RATS The amounts of radioactivity recovered from the faeces of the six animals which received test doses of 10, 50, 100, and 500 mg. of ^{131}I-labelled triolein are given in Table I; the mean excretion of radioactivity after each dose is shown in Fig. 1. The actual amounts of ^{131}I-labelled fat absorbed from the different doses, calculated by assuming that the radioactive material not recovered in the faeces was absorbed, are given in Table II; the mean absorption at each dose level is illustrated in Fig. 2.

TABLE I

FAECAL EXCRETION OF ^{131}I-LABELLED TRIOLEIN (% OF DOSE) AFTER INTRAGASTRIC DOSES OF 10 TO 500 MG.

Rat No.	*Amount Fed (mg.)*			
	10	*50*	*100*	*500*
1	11·0	8·3	14·8	30·3
2	11·0	11·6	13·9	22·8
3	15·6	9·6	14·3	23·0
4	10·5	14·6	14·8	23·8
5	13·5	12·5	20·6	22·4
6	9·2	9·4	18·3	23·1
Mean ± S.D. =	11·8 ± 2·33	11·0 ± 2·34	16·0 ± 2·7	24·2 ± 3·01

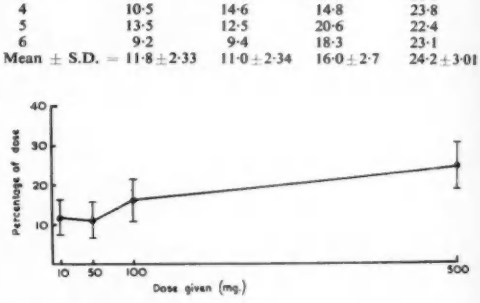

FIG. 1. *Mean percentage of administered dose (± 2 S.D.) excreted in the faeces of six rats given 10, 50, 100, or 500 mg. of ^{131}I-labelled triolein.*

When 10 mg. of ^{131}I-labelled triolein was given, the percentage of this dose excreted in the faeces ranged from 9·2 to 15·6 (mean 11·8%). Similar amounts were excreted after doses of 50 mg., but the percentage excretion of the dose increased when doses of 100 or 500 mg. were fed, the faecal excretion ranging from 13·9 to 20·6 (mean 16·0%) when 100 mg. was given, and from 22·4 to 30·3 (mean 24·2%) after the 500 mg. dose. Although the percentage of the dose excreted in the faeces was greater when the larger doses were given, there was a progressive and linear increase in the actual amount of ^{131}I-labelled fat which was absorbed when the concentration of the dose was increased (Table II, Fig. 2).

TABLE II

CALCULATED ABSORPTION[1] OF ^{131}I-LABELLED TRIOLEIN (MG.) FROM INTRAGASTRIC DOSES OF 10 TO 500 MG.

Rat No.	*Amount Fed (mg.)*			
	10	*50*	*100*	*500*
1	8·9	45·8	85·2	348·0
2	8·9	44·2	86·1	386·0
3	8·4	45·2	85·7	385·0
4	8·9	42·7	85·2	381·0
5	8·6	43·7	79·4	388·0
6	9·1	45·3	81·7	384·0
Mean =	8·8	44·5	83·9	379·0

[1]The absorption of ^{131}I-labelled triolein has been calculated by assuming that the radioactivity not recovered in the faeces (Table I) was absorbed.

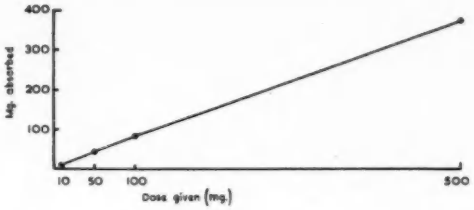

FIG. 2. *Mean absorption of labelled fat by six rats given doses of 10, 50, 100, or 500 mg. of ^{131}I-labelled triolein.*

DISTRIBUTION OF RADIOACTIVITY IN STOMACH AND INTESTINES DURING ABSORPTION The percentages of the different doses found in the stomach and different segments of the small intestine and the colon of the four groups of rats killed at varying time intervals after receiving 10 to 500 mg. of ^{131}I-labelled triolein are given in Tables III (10 mg.), IV (50 mg.), V (100 mg.), and VI (500 mg.). From these results the percentage gastric emptying and percentage absorption of the test doses have been calculated for each animal.

Gastric emptying The percentages of gastric emptying in the four groups of animals are detailed in Tables III to VI. The mean gastric emptying in the three rats killed at each time after the different doses is shown in Fig. 3.

At quarter of an hour after feeding, there was no significant difference in the percentages of the different doses which had left the stomach, the mean percentage gastric emptying ranging from 41·2 to 50·3% (Fig. 3). By half and one hour, the percentages of gastric emptying had increased after all four

TABLE III

PERCENTAGE OF 10 MG. DOSE OF ^{131}I-LABELLED TRIOLEIN IN STOMACH AND INTESTINES OF RATS KILLED AT INTERVALS OF QUARTER OF AN HOUR TO FOUR HOURS

Rat No.	*Time Killed (hr.)*	*Stomach*	*Small Intestine*				*Colon*	*Total*	*%[1] Absorbed*	*Gastric[2] Emptying*
			1	*2*	*3*	*4*				
7	¼	39·5	14·0	33·4	5·2	0·0	0·0	92·1	7·9	61·5
8	¼	45·0	25·0	13·8	3·1	0·1	0·2	87·3	12·7	55·0
9	¼	64·5	20·2	12·0	0·6	0·3	0·3	97·9	2·1	35·5
	Mean =	49·7	19·7	19·7	3·0	0·1	0·2	92·4	7·6	50·3
10	½	22·3	6·8	9·7	13·9	0·0	0·0	52·7	47·3	77·7
11	½	17·2	26·6	21·4	0·2	0·2	1·3	66·9	33·1	82·8
12	½	31·2	11·4	10·6	10·6	0·2	0·7	64·7	35·3	68·8
	Mean =	23·6	14·9	13·9	8·2	0·1	0·7	67·4	32·6	76·4
13	1	36·8	3·1	4·5	6·5	0·5	0·4	51·8	48·2	63·2
14	1	29·6	5·5	3·3	8·1	3·4	0·5	50·4	49·6	70·4
15	1	29·5	4·6	4·2	11·7	0·2	1·5	51·7	48·3	70·5
	Mean =	31·9	4·4	4·0	8·8	1·4	0·8	51·3	48·7	68·1
16	1¼	28·5	5·8	3·9	10·6	0·5	0·6	49·9	50·1	71·5
17	1¼	10·4	6·8	7·4	9·8	0·3	1·0	35·7	64·3	89·6
18	1¼	25·5	5·9	4·7	6·0	4·6	1·2	47·9	52·1	74·5
	Mean =	21·5	6·2	5·3	8·8	1·8	0·9	44·5	55·5	78·5
19	2	20·0	4·2	2·3	7·5	1·9	0·8	34·7	65·3	80·0
20	2	16·7	3·7	2·5	5·9	2·9	4·8	37·5	62·5	83·3
21	2	17·4	4·1	7·7	4·7	1·1	0·7	35·7	64·3	82·6
	Mean =	18·0	4·0	4·2	6·0	2·0	2·1	36·3	63·7	82·0
22	3	10·6	1·6	2·2	1·3	4·8	3·9	24·0	76·0	89·4
23	3	5·4	1·0	1·2	1·2	5·7	7·7	22·2	77·8	94·6
24	3	19·4	1·1	1·0	0·6	4·4	5·2	31·7	68·3	80·6
	Mean =	11·8	1·2	1·5	0·8	4·9	5·6	25·9	74·1	88·2
25	4	17·6	0·8	1·6	0·8	7·9	3·1	31·8	68·2	82·4
26	4	15·8	0·9	1·8	1·8	8·6	5·7	34·6	65·4	84·2
27	4	9·3	1·1	2·3	0·9	4·6	5·9	24·1	75·9	90·7
	Mean =	14·3	0·9	1·9	1·2	7·0	4·9	30·2	69·8	85·7

[1]Percentage absorption was calculated by assuming that the material not recovered had been absorbed.
[2]Percentage gastric emptying is the amount of material not recovered from the stomach.

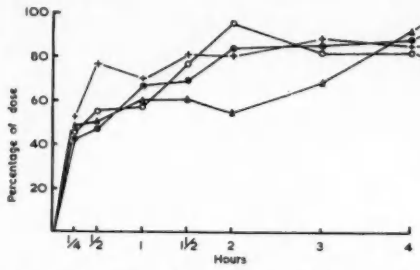

FIG. 3. *Mean gastric emptying at intervals of quarter hour to four hours after feeding doses of 10* (+——+), *50* (○——○), *100* (●——●), *or 500* (▲——▲) *mg. of* ^{131}*I-labelled triolein.*

doses. Between one and three hours, however, gastric emptying was invariably less complete after the 500 mg. dose than after doses of 10, 50, or 100 mg. of labelled fat. Although more than 80% of doses of 10 to 100 mg. of fat had left the stomach at two and three hours, for instance, the mean gastric emptying after doses of 500 mg. was only 54·2% at two hours and 69·6 at three hours (Fig. 3). By four hours, the degree of gastric emptying was similar in the four groups of animals.

Rates of absorption The total amounts of radioactivity recovered from the intestinal tracts of the four groups of rats are also given in Tables III to VI. The absorption by the different rats killed at each time interval, calculated by assuming that the radioactivity not recovered had been absorbed, is given in these Tables. The mean amounts absorbed by the three rats killed at each time after the four different doses are shown in Figs. 4 and 5.

The rates of absorption of the different test doses varied according to the amount of triolein which was given. In terms of the percentage of each dose absorbed, absorption was most rapid after feeding the 10 mg. dose and became progressively slower as the amount of fat in the test dose was increased (Fig. 4). If these results are expressed as the absolute amount of fat absorbed, on the other hand, it can be seen that the more fat was given, the greater was the actual amount of fat absorbed in a given time (Fig. 5).

Distribution of radioactivity in small intestine The percentages of the administered radioactivity found in the four segments of the small intestine of each rat are given in Tables III to VI. The mean amounts in the different segments of the three rats killed at each time after the various doses are shown in Fig. 6.

TABLE IV

PERCENTAGE OF 50 MG. DOSE OF ^{131}I-LABELLED TRIOLEIN IN STOMACH AND INTESTINES OF RATS KILLED AT INTERVALS OF QUARTER OF AN HOUR TO FOUR HOURS

Rat No.	*Time Killed (hr.)*	*Stomach*	*Small Intestine*				*Colon*	*Total*	*%[1] Absorbed*	*Gastric[2] Emptying*
			1	*2*	*3*	*4*				
28	¼	25·7	26·8	39·6	5·3	0·0	1·2	98·6	1·4	74·3
29	¼	73·7	7·2	11·9	0·0	0·0	0·0	92·8	7·2	26·3
30	¼	62·7	10·2	25·2	0·0	0·0	0·0	98·1	1·9	37·3
	Mean =	54·0	14·7	25·6	1·8	0·0	0·4	96·5	3·5	46·0
31	½	52·3	16·0	15·2	0·9	0·2	0·8	85·4	14·6	47·7
32	½	37·1	6·3	9·4	19·2	0·3	0·8	73·1	26·9	62·9
33	½	46·7	3·0	32·2	1·7	0·0	0·0	83·6	16·4	53·3
	Mean =	45·4	8·4	18·9	7·3	0·2	0·5	80·7	19·3	54·6
34	1	35·4	8·7	5·1	6·4	15·0	0·5	71·1	28·9	64·6
35	1	30·6	5·9	7·3	12·2	0·0	0·2	56·2	43·8	69·4
36	1	61·5	7·1	2·1	13·4	0·5	0·2	84·8	15·2	38·5
	Mean =	42·5	7·2	4·8	10·7	5·2	0·3	70·7	29·3	57·5
37	1½	35·0	7·1	4·3	4·5	12·2	1·2	64·3	35·7	65·0
38	1½	19·5	2·3	2·9	21·8	0·3	0·6	47·4	52·6	80·5
39	1½	14·2	4·3	16·4	22·3	0·3	0·5	58·0	42·0	85·8
	Mean =	22·9	4·6	7·9	16·2	4·3	0·8	56·6	43·4	77·1
40	2	15·6	7·7	13·3	8·3	0·5	0·9	46·3	53·7	84·4
41	2	8·3	1·4	3·4	8·2	11·8	0·6	39·7	66·3	91·7
42	2	18·6	2·9	12·2	12·9	0·4	0·5	47·5	52·5	81·4
	Mean =	14·2	4·0	9·6	9·8	4·3	0·7	42·5	57·5	95·8
43	3	24·5	2·6	1·9	1·6	2·0	8·8	41·6	58·4	76·5
44	3	11·8	2·8	2·0	4·4	3·0	11·0	35·0	65·0	88·2
45	3	13·2	2·1	1·7	1·0	8·4	2·6	29·0	71·0	86·8
	Mean =	16·5	2·7	2·9	2·3	4·5	7·5	35·2	64·8	83·5
46	4	6·8	4·5	4·3	0·6	1·5	8·6	26·3	73·7	93·2
47	4	13·6	1·4	1·4	1·4	1·5	7·9	27·2	72·8	86·4
48	4	27·9	2·2	2·2	1·1	2·5	8·9	34·8	63·2	72·1
	Mean =	16·1	2·7	2·5	1·0	2·8	8·5	30·1	69·9	83·9

[1]Percentage absorption was calculated by assuming that the material not recovered had been absorbed.
[2]Percentage gastric emptying is the amount of material not recovered from the stomach.

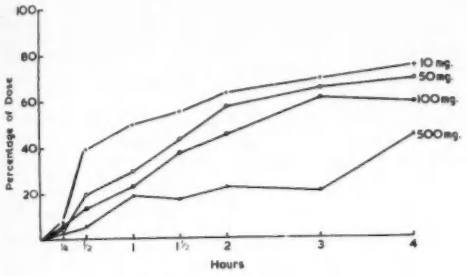

FIG. 4. *Mean percentage absorption of doses of 10 (+——+), 50 (○——○), 100 (●——●), or 500 (▲——▲) mg. of fat at intervals of quarter of an hour to four hours after feeding.*

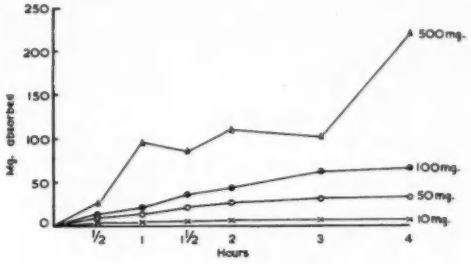

FIG. 5. *Mean amounts of fat absorbed (mg.) at intervals of quarter hour to four hours after feeding doses of 10 (×——×), 50 (○——○), 100 (●——●), or 500 (▲——▲) mg. of ^{131}I-labelled triolein.*

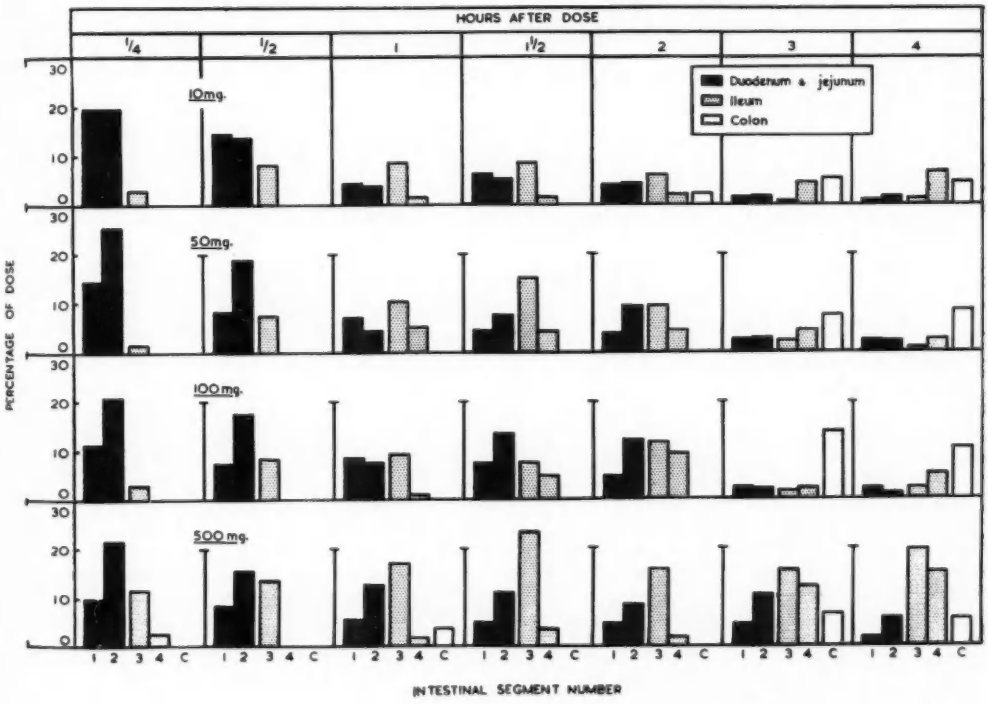

FIG. 6. *Distribution of radioactive material in the different segments of the small intestine and in the colon of rats killed at intervals of quarter of an hour to four hours after feeding doses of 10, 50, 100, or 150 mg. of ^{131}I-labelled triolein. The intestinal segments were numbered as follows: (1) Duodenum and upper jejunum, (2) lower jejunum, (3) upper ileum, (4) lower ileum. C indicates the colon.*

When 10 mg. of ^{131}I-labelled triolein was given, the mean percentage of the dose found in each of the proximal two segments at quarter of an hour was 19·7% (Fig. 6, Table III). The radioactivity in these segments fell gradually during the next one to three hours as absorption took place (Fig. 4), so that at three and four hours no more than 1·9% of the administered radioactivity was present.

At the same time, the mean radioactivity in the third segment rose from 3·0% at quarter of an hour to 8·8% at one and one and a half hours. It then fell to 0·8 and 1·2% at three and four hours. The fourth segment contained only small amounts of radioactivity until three and four hours, at which times the mean radioactivity in this segment was 4·9 and 7·0% respectively (Fig. 6, Table III).

When the dose was increased to 50 mg., the mean percentage of the radioactivity present in the first segment at quarter of an hour was less than after 10 mg., being 14·7%. At this time, more radioactivity (25·6%) was found in the second segment. The radioactivity in these two segments fell more slowly than when 10 mg. was given, 9·6% of the dose being still present in the second segment at two hours (Fig. 6, Table IV). By three and four hours, as absorption proceeded, less than 2·9% was present in either of segments 1 and 2.

The mean radioactivity in the third segment was only 1·8% at quarter of an hour but rose to a peak of 16·2% at one and a half hours. It then fell gradually to 2·3% at three hours and 1% at four hours (Fig. 6, Table IV).

The fourth segment contained little or no radioactivity at quarter or half an hour but between 4 and 5% of the administered radioactivity was found in this segment between one and three hours.

After the dose of 100 mg. the mean percentage radioactivity in the different segments was essentially similar to that after a dose of 50 mg. However, a greater proportion of the administered radioactivity was present in segments 3 and 4 at two hours than when either 10 or 50 mg. was given (Fig. 6, Table V).

When a dose of 500 mg. was given, the percentage of the radioactivity recovered from segments 1 and 2 between a quarter of an hour and two hours was similar to that after 50 or 100 mg. However, the radioactivity in these segments fell more slowly than after the smaller doses, for segment 1 still contained as much as 4·6% and segment 2, 10·4% at three hours, at which time the mean radioactivity in these segments was invariably less than 2 to 3% after doses of 10 to 100 mg. (Fig. 6, Table VI).

The mean radioactivity in the third segment was

TABLE V

PERCENTAGE OF 100 MG. DOSE OF ^{131}I-LABELLED TRIOLEIN IN STOMACH AND INTESTINES OF RATS KILLED AT INTERVALS OF QUARTER OF AN HOUR TO FOUR HOURS

Rat No.	*Time Killed (hr.)*	*Stomach*	*Small Intestine*				*Colon*	*Total*	*%[1] Absorbed*	*Gastric[2] Emptying*
			1	*2*	*3*	*4*				
49	¼	71·0	9·0	11·2	7·6	0·0	0·1	98·9	1·1	29·0
50	¼	56·0	83·0	23·3	1·5	0·0	0·4	94·2	5·8	44·0
51	¼	49·3	13·2	28·6	0·0	0·0	0·0	91·1	8·9	50·7
	Mean =	58·8	11·7	21·0	3·0	0·0	0·2	94·7	5·3	41·2
52	½	71·7	4·5	6·1	13·7	0·5	0·7	97·2	2·8	28·3
53	½	44·5	7·3	22·6	9·3	0·0	0·6	84·3	15·7	55·5
54	½	40·2	10·3	24·6	1·0	0·3	0·4	76·8	23·2	59·8
	Mean =	52·1	7·4	17·8	8·3	0·3	0·6	86·1	13·9	47·9
55	1	59·5	3·1	6·9	9·1	1·9	1·1	79·6	20·4	40·5
56	1	37·2	11·7	7·4	9·2	1·0	0·0	66·5	33·5	62·8
57	1	57·0	11·0	8·7	9·9	0·0	0·3	86·9	13·1	63·0
	Mean =	51·2	8·6	7·7	9·4	1·0	0·5	77·7	22·3	68·8
58	1½	48·9	1·9	3·4	9·0	9·8	0·4	73·4	26·6	51·1
59	1½	28·2	10·8	20·4	7·3	0·3	0·7	67·7	32·3	71·8
60	1½	16·0	10·2	17·0	7·2	4·9	0·9	56·2	43·8	84·0
	Mean =	31·0	7·6	13·6	7·8	5·0	0·7	62·4	37·6	69·0
61	2	7·4	1·5	8·3	18·8	22·3	0·6	59·9	40·1	92·6
62	2	12·9	6·9	19·1	7·0	1·9	1·3	49·1	50·9	87·1
63	2	28·8	6·7	8·8	9·5	3·5	0·9	58·2	41·8	71·2
	Mean =	16·4	5·0	12·1	11·8	9·3	0·9	55·7	44·3	83·6
64	3	7·3	1·9	3·0	1·5	2·5	11·4	27·6	72·4	92·7
65	3	23·5	3·9	2·3	2·5	3·1	13·2	48·5	51·5	76·5
66	3	16·2	1·6	1·6	1·8	1·7	17·4	40·3	59·7	83·8
	Mean =	15·7	2·5	2·3	1·9	2·4	14·0	48·8	61·2	84·3
67	4	5·6	2·3	1·4	2·4	2·3	13·8	27·8	72·2	94·4
68	4	16·6	1·7	1·1	2·8	8·6	6·8	37·6	62·4	83·4
69	4	12·4	2·3	1·1	1·8	5·2	11·8	34·6	65·4	87·6
	Mean =	11·5	2·1	1·2	2·3	5·4	10·8	33·3	66·7	88·5

[1]Percentage absorption was calculated by assuming that the material not recovered had been absorbed.
[2]Percentage gastric emptying is the amount of material not recovered from the stomach.

TABLE VI

PERCENTAGE OF 500 MG. DOSE OF ^{131}I-LABELLED TRIOLEIN IN STOMACH AND INTESTINES OF RATS KILLED AT INTERVALS OF QUARTER OF AN HOUR TO FOUR HOURS

Rat No.	*Time Killed (hr.)*	*Stomach*	*Small Intestine*				*Colon*	*Total*	*%[1] Absorbed*	*Gastric[2] Emptying*
			1	*2*	*3*	*4*				
70	¼	44·7	13·6	6·9	23·1	8·3	0·0	96·6	3·4	55·3
71	¼	56·8	10·3	24·8	7·1	0·0	0·0	98·0	2·0	43·2
72	¼	55·6	6·0	32·8	4·6	0·0	0·0	99·0	1·0	44·4
	Mean =	52·0	10·0	21·5	11·6	2·8	0·0	97·9	2·1	48·0
73	½	52·0	11·0	12·2	16·6	0·8	0·0	92·6	7·4	48·0
74	½	60·1	5·6	13·0	11·3	0·4	0·3	91·7	8·3	39·9
75	½	42·7	8·5	24·4	12·9	0·0	0·0	98·5	1·5	57·3
	Mean =	51·6	8·4	16·5	13·6	0·4	0·1	94·3	5·7	48·4
76	1	36·0	7·3	8·6	21·4	0·8	3·6	77·7	22·3	64·0
77	1	31·5	3·8	16·7	19·2	4·6	6·9	82·7	17·3	68·5
78	1	54·5	5·5	12·8	10·5	0·0	0·0	83·3	16·7	45·5
	Mean =	40·7	5·5	12·7	17·0	1·8	3·5	82·2	18·8	59·3
79	1¼	35·2	5·6	14·6	20·1	3·5	0·3	79·3	20·7	64·8
80	1¼	40·2	2·7	7·6	33·0	6·6	0·6	90·7	9·3	59·8
81	1¼	43·3	6·3	10·4	17·5	0·0	0·1	77·6	22·4	56·7
	Mean =	39·6	4·9	10·9	23·5	3·4	0·3	82·5	17·5	60·4
82	2	66·1	2·5	3·4	4·6	3·9	2·0	82·5	17·5	43·9
83	2	30·7	7·3	12·0	24·8	1·5	0·6	76·9	23·1	69·3
84	2	40·7	4·6	10·5	17·6	0·0	0·2	73·6	26·4	59·3
	Mean =	45·8	4·8	8·6	15·7	1·8	0·9	77·7	22·3	54·2
85	3	28·2	1·2	12·7	38·2	9·7	0·5	85·5	14·5	71·8
86	3	35·0	3·1	3·6	7·7	10·5	19·4	79·3	20·7	65·0
87	3	28·0	9·5	15·0	4·5	15·9	0·9	73·8	26·2	72·0
	Mean =	30·4	4·6	10·4	15·1	12·0	6·9	79·5	20·5	69·6
88	4	5·8	1·7	3·1	28·9	19·7	0·8	59·2	40·8	94·2
89	4	8·3	2·3	5·0	9·4	12·9	14·6	52·5	47·5	91·7
90	4	9·6	1·6	9·5	21·6	12·4	1·1	55·8	44·2	90·4
	Mean =	7·9	1·9	5·9	19·9	15·0	5·5	55·8	44·2	92·1

[1]Percentage absorption was calculated by assuming that the material not recovered had been absorbed.
[2]Percentage gastric emptying is the amount of material not recovered from the stomach.

always greater than when doses of 10, 50, or 100 mg. were given. It rose from 11·6% at a quarter of an hour to a maximum of 23·5% at one and a half hours, and remained as high as 15·7, 15·1, and 19·9% at two, three, and four hours (Fig. 6, Table VI) when less than 3% was recovered from this segment after the smaller doses.

The fourth segment contained only small amounts of radioactivity until three and four hours, at which times the mean radioactivity recovered from this segment was 12·0 and 15·0% respectively. These amounts were also greater than were found in the fourth segment after the smaller doses.

Radioactivity in the colon Insignificant amounts of radioactivity were recovered from the colon until two to three hours after the doses were given (Fig. 6, Tables III to VI). At three and four hours, the colonic radioactivity was invariably less than the amounts excreted in the faeces after similar doses were given to intact rats (Table I).

DISCUSSION

AMOUNTS OF FAT ABSORBED FROM INCREASING DOSES These results show that when increasing doses of ^{131}I-labelled fat are given to rats, there is a progressive increase in the percentage of the dose excreted in the faeces (Table I, Fig. 1). In terms of the amount of fat absorbed, however, there is a progressive increase in absorption as increasing doses of ^{131}I-labelled triolein are given (Table II, Fig. 2).

SITES OF FAT ABSORPTION The sites of absorption of the various doses varied according to the amount fed. Since absorption invariably began soon after feeding (Fig. 4), it seemed likely that the labelled material was absorbed first from the upper intestine regardless of the amount of fat given. This was confirmed by the studies of the distribution of radioactivity in the different segments of the small intestine during absorption (Fig. 6). Since much of the radioactivity in the proximal two segments disappeared during the first hour after feeding without subsequently appearing in similar amounts in the more distal segments, it is evident that the jejunum absorbed considerable amounts of fat. The importance of the ileum (segments 3 and 4) in fat absorption depended on the amount of fat given, for the radioactivity reaching the distal two segments was proportional to the amount of labelled fat given, the greatest amounts being recovered from the ileum after the largest doses (Fig. 6). However, the

recovery of radioactive material from the ileum did not necessarily indicate that absorption occurred there. Although as much as 10% of a dose of 10 mg. of ^{131}I-labelled triolein reached the ileum (segments 3 and 4, Table III, Fig. 6), it could not be assumed that this material was absorbed from that area. The mean faecal excretion after a dose of 10 mg. of fat by an intact rat was 11·8% (Table I, Fig. 1), and the 10% recovered from the ileum after a dose of 10 mg. was therefore likely to be merely in transit to the colon for subsequent elimination in the faeces. When larger amounts of fat were fed, however, the percentages which reached the distal intestine were greater than were excreted in the faeces of intact rats given similar doses (Figs. 1 and 6), indicating that part of these larger doses must have been absorbed in the ileum.

An approximate estimate of the amount of each dose which was absorbed in the ileum has been obtained by subtracting the percentage of the dose excreted in the faeces by intact rats given the various doses (Table I, Fig. 1) from the maximum percentages of each dose recovered from the ileum together with the colon at any one time (Tables III to VI, Fig. 6). If the amount of fat absorbed in the ileum is then subtracted from the amount shown to be absorbed from the equivalent doses (Table II, Fig. 2), a similar approximation of jejunal absorption may be derived. The results of these estimations, set out in Table VII, indicate that when 10 mg. of ^{131}I-labelled fat was given absorption was almost entirely in the jejunum (Table VII). Larger doses of fat were also absorbed to a great extent in the jejunum, which responded to an increased dietary load by an increased absorption, but at the same time increasing amounts of fat escaped absorption in the proximal segments and therefore passed into the ileum; an increasing proportion was then absorbed there (Table VII). The ileum of the rat, however, appears to be less efficient at absorbing fat than the jejunum, for much of the labelled material which reached the ileum passed on into the colon and was excreted in the faeces (Tables I and VII). Dawson and Isselbacher (1960), studying the esterification of palmitate-1-C^{14} by rat intestinal mucosa *in vitro*, have shown that cell-free homogenates prepared from the ileum are a quarter to a fifth as active as those from the jejunum. The results given in this paper demonstrate an interesting parallel *in vivo* to their observations.

TABLE VII

CALCULATED ABSORPTION OF ^{131}I-LABELLED TRIOLEIN FROM JEJUNUM AND ILEUM

		Amount Fed (mg.)			
		10	*50*	*100*	*500*
Mean faecal excretion (% of dose)[1]		11·8	11·0	16·0	24·2
Maximum mean radioactivity in ileum plus colon (% of dose)[2]		13·1	21·3	22·1	40·5
Calculated absorption from ileum	% of dose	1·3	10·3	6·1	16·3
	mg.	0·1	5·1	6·1	81·0
Calculated absorption from jejunum	% of dose	86·9	78·7	77·9	59·6
	mg.	8·7	39·4	77·9	298·0

[1]Data from Table I.
[2]Data from Tables III, IV, V, and VI.

The observation that fat may be absorbed in increasing amounts from both the jejunum and the ileum when the dietary load is increased provides a rational explanation for the unlimited capacity of the small intestine to absorb fat (Fig. 2). The absorption of the largest doses may also be facilitated by slower gastric emptying after such doses (Fig. 3), which serves to reduce the amount of fat delivered to the intestine at any one time.

RELATIONSHIP BETWEEN RATES AND SITES OF ABSORPTION If absorption of a substance begins in the upper jejunum, the site of its absorption depends on the relationship between its absorption rate and the upper intestinal motility (Parkins *et al.*, 1960). Glucose or iodide, for instance, are absorbed rapidly and their absorption occurs in the proximal half of the small intestine of the rat (Reynell and Spray, 1956; Parkins *et al.*, 1960). ^{131}I-labelled albumin, however, is absorbed more slowly; the rapid motility of the upper small intestine propels it more distally before absorption is complete and some absorption therefore also occurs in the ileum (Parkins *et al.*, 1960).

There appears to be a similar relationship between absorption rate and the site of absorption of different doses of fat. The 10 mg. dose of fat, for instance, was absorbed relatively rapidly (Fig. 4) and absorption therefore occurred in the jejunum (Table VII). A 500 mg. dose, however, was absorbed more slowly (Fig. 4) and a greater proportion was therefore propelled as far as the ileum before absorption was complete.

PREVIOUS OBSERVATIONS ON SITE OF FAT ABSORPTION The results given in this paper are in keeping with previous observations which have suggested that the jejunum plays a major part in fat absorption (Borgström *et al.*, 1957; Favarger and Gerlach, 1953; Frazer, 1943; Turner, 1958). Other workers have claimed that in the rat maximal absorption of ^{131}I-labelled olive oil occurs in the third quarter of the small intestine, an area said to be specifically adapted for this purpose (Benson *et al.*, 1956). Fig. 6

illustrates a possible reason for this conclusion. These workers killed their rats only at two hours and later after the administration of labelled fat, and measured the radioactivity in the mucosa of different segments of the small intestine. When rats are killed at two hours and later after the test doses, most of the radioactive material is likely to be recovered from the ileum, for most of the jejunal fat has already been absorbed in this time (Fig. 6).

SUMMARY

The faecal excretion of radioactivity was measured in rats given doses of 10, 50, 100, or 500 mg. of ^{131}I-labelled triolein. As the dose was increased, the percentage of the dose excreted in the faeces became greater but there was a progressive increase in the amount of fat which was absorbed.

The sites of absorption of the ^{131}I-labelled triolein varied according to the dose given. A dose of 10 mg. was absorbed relatively rapidly and absorption occurred in the jejunum. Larger doses, however, were absorbed more slowly and the motility of the upper intestine propelled the fat more distally before absorption was complete; absorption therefore also occurred in the ileum when the larger doses were given.

The increase in absorption of the labelled triolein which occurred when increasing doses were given was achieved first by an enhanced jejunal absorption in response to an increased dietary load, and secondly by an increased absorption in the ileum.

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REFERENCES

Balint, J., Pendower, J., and Ramsey, N. W. (1960). The stability of radio-iodinated olive oil. *Clin. Sci.*, **19**, 321-325.

Benson, J. A. Jr., Chandler, G. N., Vansteenhuyse, F. E., and Gagnon, J. O. (1956). Studies concerning the site of fat absorption in the small intestine of the rat. *Gastroenterology*, **30**, 53-61.

Booth, C. C. (1958). *An Experimental and Clinical Study of the Intestinal Absorption of Vitamin B_{12}*. M.D. Thesis, St. Andrews University.

——, and Mollin, D. L. (1959). The site of absorption of vitamin B_{12} in man. *Lancet*, **1**, 18-21.

Borgström, B., Dahlqvist, A., Lundh, G., and Sjövall, J. (1957). Studies of intestinal digestion and absorption in the human. *J. clin. Invest.*, **36**, 1521-1536.

Cummins, A. J., and Jussila, R. (1955). Comparison of glucose absorption rates in the upper and lower human small intestine. *Gastroenterology*, **29**, 982-992.

Dawson, A. M., and Isselbacher, K. J. (1960). The esterification of palmitate-1-C^{14} by homogenates of intestinal mucosa. *J. clin. Invest.*, **39**, 150-160.

Favarger, P., and Gerlach, J. (1953). Variations dans le mode de résorption des différentes graisses et acides gras chez le rat. *Helv. physiol. pharmacol. Acta*, **11**, 239-250.

Frazer, A. C. (1943). Differentiation in the absorption of olive oil and oleic acid in the rat. *J. Physiol. (Lond.)*, **102**, 306-312.

Kremen, A. J., Linner, J. H., and Nelson, C. H. (1954). An experimental evaluation of the nutritional importance of proximal and distal small intestine. *Ann. Surg.*, **140**, 439-448.

Lewis, S. M., and Porter, I. H. (1960). Erythrocyte survival in rheumatoid arthritis. *Ann. rheum. Dis.*, **19**, 54-58.

Lubran, M., and Pearson, J. D. (1958). A screening test for steatorrhoea using ^{131}I-labelled triolein. *J. clin. Path.*, **11**, 165-169.

Parkins, R. A., Dimitriadou, A., and Booth, C. C. (1960). The rates and sites of absorption of ^{131}I-labelled albumin and sodium ^{131}I in the rat. *Clin. Sci.*, **19**, 595-604.

Reynell, P. C., and Spray, G. H. (1956). The simultaneous measurement of absorption and transit in the gastro-intestinal tract of the rat. *J. Physiol. (Lond.)*, **131**, 452-462.

Turner, D. A. (1958). The absorption, transport, and deposition of fat: application of a new method for the determination of ^{131}I-lipid activity in dogs and man. *Amer. J. dig. Dis.*, n.s., **3**, 594-640, 682-708.

Van Handel, E., and Zilversmit, D. B. (1958). Limitation of radioiodine as a label for fat. *J. lab. clin. Med.*, **52**, 831-839.

Veall, N., and Baptista, A. M. (1954). A multi-tube gamma counting apparatus for small samples. *Brit. J. Radiol.*, **27**, 198-199.

Verzár, F., and McDougall, E. J. (1936). *Absorption from the Intestine*. Longmans, Green, London.

Gut, 1961, **2**, 32

The histamine test meal in the rat

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SYNOPSIS A histamine test meal is described for the estimation of the secretion of gastric acid and pepsin in the unanaesthetized intact rat.

The stomach of the rat secreted pepsin and a small amount of acid in response to the test meal of hypertonic sucrose. Stimulation with histamine produced a pronounced rise in acid secretion but the output of pepsin remained unchanged. The increase in acid secretion was correlated in the individual rat with a rise in the volume of gastric secretion. Histamine phosphate in a dose of 50 mg./kg. body weight produced maximal stimulation of gastric acid secretion and was free from toxic effects. Choline chloride, in a dose of 126·5 mg./kg. body weight, had no effect on the volume of gastric secretion and it was ineffective as a stimulant of either acid or pepsin secretion.

There are conflicting reports in the literature on the effectiveness of histamine and choline as gastric stimulants in the rat. Roe and Dyer (1939), Friedman (1943), and Komarov, Shay, Rayport, and Fels (1944), using rats in which the pylorus had been ligated, reported that histamine had little if any stimulating effect on the volume and acidity of gastric secretion. Using a similar but improved technique, Kyle and Welbourn (1956) reported that histamine given in doses of 3·6 mg./kg. body weight per hour produced a 50% increase in both the volume and acidity of gastric secretion in the rat. Lane, Ivy, and Ivy (1957) found that histamine had a pronounced effect on the acid secretion of the rat with a gastric fistula; similar results were obtained by Ghosh and Schild (1955).

In 1944, Komarov and his colleagues, using the anaesthetized rat in which the pylorus had been ligated, reported that choline was an effective stimulant of both gastric acid and pepsin secretion. They stated, 'We have been so impressed with the relatively uniform effect of choline chloride upon gastric scretion that we have adopted it as a routine agent for testing secretory function in the rat'. To the best of our knowledge no other studies of the efficacy of choline as a gastric stimulant have been reported.

A substantial improvement in the method of studying gastric acid secretion was made by Thornton and Clifton (1959), who devised a test meal technique suitable for the unanaesthetized intact rat. This method involves instilling a test meal containing sucrose and phenol red into an animal's stomach with a catheter. The gastric contents are aspirated after 45 minutes, and the levels of acid and phenol red are measured. The concentration of phenol red is used to calculate both the volume of gastric secretion and the amount of acid that passed through the pylorus during the test period. Using this method they found histamine phosphate, in a dose of 12 mg./kg. body weight, an effective stimulant of gastric acid secretion.

In the present investigation the procedure described by Thornton and Clifton (1959) has been modified in several ways and the method has been expanded to include the estimation of gastric pepsin. The technique has been used to reassess the effects of histamine and choline on the volume and acidity of gastric secretion and to determine the effect of these drugs on gastric motility and the secretion of pepsin. The investigation was carried out in two parts. First, a study was made of the effect of graded amounts of histamine on gastric function in order to determine a dose that would give a maximal stimulus. Secondly, the effect of this dose of histamine was compared with injections of saline and choline chloride.

METHODS

RATS AND THEIR CARE Female rats of the Wistar strain weighing about 250 g. were used. They were kept in individual cages with wide-mesh floors. The animals were deprived of food for 48 hours before the test and fluid was provided in the form of distilled water which contained 10% sucrose, 0·25% NaCl, and 0·25% KCl.

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FIG. 1. *Position of rat for intubation.*

TEST MEAL The test meal used in this study was similar to that described by Thornton and Clifton (1959) except that the final *p*H of the solution was adjusted to 5·0 rather than 6·5, since pepsin is more stable at the lower *p*H (Linde, 1950). Only 5 ml. of the test meal was administered to each rat as larger volumes tended to exceed the capacity of the stomach. Thornton and Clifton assumed that phenol red was not absorbed from the stomach of the rat and could be quantitatively recovered. In nine rats in which the pylorus had been ligated, we found that on the average 97% of phenol red could be recovered from the stomach two minutes after instillation and 94% after 45 minutes. Although some loss appears to occur it is not sufficient to make the use of phenol red impracticable.

INTUBATION OF ANIMALS AND ADMINISTRATION OF TEST MEAL The simple method of intubation described by Thornton and Clifton is only applicable to animals that have been repeatedly intubated. Since this was impracticable in the present study, a method was devised for restraining and intubating the animals. The rat was held by an attendant as shown in Fig. 1. The fore-paws and skin over the abdomen were grasped firmly with the left hand while the animal's head was held between the thumb and index finger of the other hand. A flattened perspex rod, with a groove on the upper edge, was used to hold the animal's mouth open and to press the tongue forward, while a rubber catheter (No. 4 English gauge), lubricated with K-Y jelly, was passed into the pharynx and pushed down the oesophagus. The stomach was washed out with aliquots of saline until a clear return was obtained. Then 5 ml. of air was injected into the stomach and this was aspirated to ensure that no fluid had been left behind. The catheter was withdrawn and the animals were permitted to recover for five minutes. The gastric secretory stimulant was given by injection and the rat was then re-intubated with a fine, No. 1 gum-elastic catheter and 5 ml. of the test meal was injected into the stomach.

The gastric contents were aspirated with a No. 4 rubber catheter 45 minutes later and the stomach was washed out with 5 ml. of saline. The specimens were collected in centrifuge tubes and these were centrifuged at 1,000 *g* for 15 minutes.

DETERMINATION OF PHENOL RED AND TOTAL ACID CONTENT Wherever the volume of the gastric contents permitted, duplicate estimations of phenol red, total acid, and pepsin were made. Phenol red was measured in the gastric contents and washout by diluting them respectively, 1 to 25 and 1 to 3, with 0·5 M-sodium phosphate buffer at *p*H 10. The optical density of the solutions was measured in a spectrophotometer at 560 mμ.

The gastric contents and washout were titrated with 0·01 N-NaOH in a combined calomel reference electrode and microtitration vessel to the initial *p*H of the test meal.

DETERMINATION OF PEPSIN A micromethod for the estimation of pepsin was developed from the method of Anson (1938). Albumin was chosen as a substrate in place of haemoglobin, because the blank solutions of the latter produced unduly high extinction coefficients. Crystalline bovine plasma albumin (3·5 g.) was dissolved in 95 ml. of distilled water and the *p*H lowered to 1·4 with HCl. The volume was made up to 100 ml., the *p*H checked again, and the solution stored in the refrigerator. Trichloracetic acid was prepared by collecting the fractions distilling between 196° and 199° C. from Analar trichloracetic acid.

For the assay the gastric contents were diluted 1 in 6 with distilled water. The gastric washout was tested without dilution. Of each solution to be tested, 0·2 ml. was added to three 1·3 × 5·3 cm. flat-bottomed tubes. One of the tubes served as the blank and 1·0 ml. of trichloracetic acid was added to it. The tubes were placed along with the albumin substrate in a water bath at 37° C. Albumin solution (0·5 ml.) was added to each tube and the contents mixed by partial inversion. After 10 minutes' incubation 1·0 ml. of 0·5 M-trichloracetic acid was added, the tubes were gently tapped, allowed to stand for 10 minutes, and then centrifuged at 1,000 *g* for 15 minutes. One millilitre of clear supernatant was removed from each tube and added to 1·0 ml. of distilled water in a 1 × 7·5 cm. test tube. The optical density of the solutions was measured at 276 mμ in silica micro-cells with a light path of 4 cm. The optical density was transformed into units of pepsin per millilitre by reference either to a standard graph or a standard tyrosine solution.

One unit of pepsin was defined as that amount of

pepsin, which when incubated with albumin substrate under the conditions specified, liberated $6{\cdot}0 \times 10^{-4}$ mEq. of tyrosine-like substances in 1 ml. of supernatant. By employing this unit the results were of similar magnitude to those obtained by the Folin method (Aitken, Spray, and Walters, 1954). In evaluating the procedure experimental evidence was obtained to show that (1) the albumin concentration is adequate for maximum enzyme activity; (2) 0·5 M-trichloracetic acid is an efficient protein precipitant in the proportions used; (3) the *p*H optimum is 1·4 to 1·5; (4) the absorption maximum of the supernatant from a digestion mixture is 276 mμ; (5) a linear relationship exists between incubation time and optional density for a period of 60 minutes; (6) for practical purposes a linear relationship exists between enzyme concentration and optical density; (7) the coefficient of variation of the method is 2%.

ACCURACY OF THE METHOD Complete aspiration of the living rat's stomach is not easy, and any fluid not aspirated after the preliminary lavage will be added to the test meal. The measurements of *p*H and pepsin in the test meal are therefore probably more reliable than those of volume of secretion and total secretion of acid.

THE OPTIMAL DOSE OF HISTAMINE IN THE RAT

Gastric analyses were performed on 24 rats of the Wistar strain which were divided into eight equal groups and given respectively, by subcutaneous injection, 0, 4, 12, 25, 50, 75, 150, and 250 mg./kg. body weight of histamine phosphate. The animals were not protected by an antihistaminic drug. One death occured in the group that received the largest dose of histamine, otherwise no toxic effects were observed. Examination of the gastric mucosa after the test meals revealed no abnormality. These results in the intact rat are in contrast to studies of Kyle and Welbourn (1956) who found that the incidence of ulceration of the gastric mucosa of rats in which the pylorus had been ligated was twice as great in animals that had received histamine as in comparable controls.

The stomach of the rat secreted pepsin and a small amount of acid in response to the test meal of hypertonic sucrose alone.

Histamine phosphate in doses up to 25 mg./kg. body weight produced a progressive rise in acid secretion; however, a further increase in the dosage was not followed by appreciable change in the output of acid (Fig. 2). Pepsin secretion, volume of gastric secretion, and gastric emptying were unaffected by histamine.

Doses of histamine ranging from 0·01 mg. to 15 mg./kg. body weight have been used in the past to stimulate gastric acid secretion in the rat (Roe and Dyer, 1939; Friedman, 1943; Kyle and Welbourn, 1956; Komarov, *et al.*, 1944; Lane, Ivy, and Ivy, 1957; Ghosh and Schild, 1955; Thornton and Clifton, 1959). When compared with the effect of histamine in the present study these levels are suboptimal. To assess adequately the capacity of the stomach to produce acid it would appear desirable to give a stimulus that induces a secretion of acid which represents the maximal parietal-cell output for that particular animal. The stimulus should be large enough to take account of individual susceptibility to histamine and it should be free from toxic effects. On the basis of our findings a dose of 50 mg./kg. body weight of histamine phosphate would appear to fulfil these criteria. The absence of toxic effects with this rather high dose is not surprising in the light of the work by Rose and Browne (1938) who clearly showed that the rat is tolerant of large amounts of histamine.

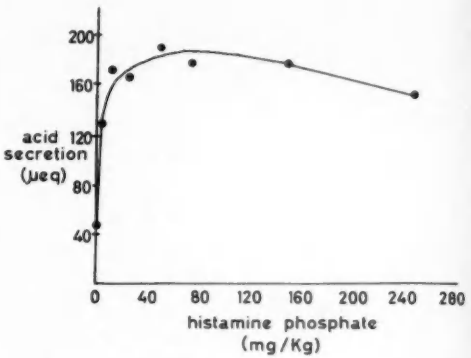

FIG. 2. *Effect of graded doses of histamine on gastric acid secretion.*

The dose of histamine required for maximum stimulation of acid secretion in the rat is about 1,000 times greater than that required in man (Kay, 1953) and about 100 to 1,000 times greater than the doses used in the dog (Bowie and Vineberg, 1935; Hanson, Grossman, and Ivy, 1948). The disagreement of previous workers on the efficacy of histamine as a stimulant of gastric secretion in the rat is undoubtedly related to the failure to give adequate amounts and to the variable and unphysiological techniques that have been employed to test gastric function.

THE EFFECT OF CHOLINE AND HISTAMINE ON GASTRIC FUNCTION IN THE RAT

Sixteen rats were divided into two groups and gastric analyses were performed after they had been given, respectively, saline and choline chloride, 126·5 mg./kg. body weight, by subcutaneous injection. The

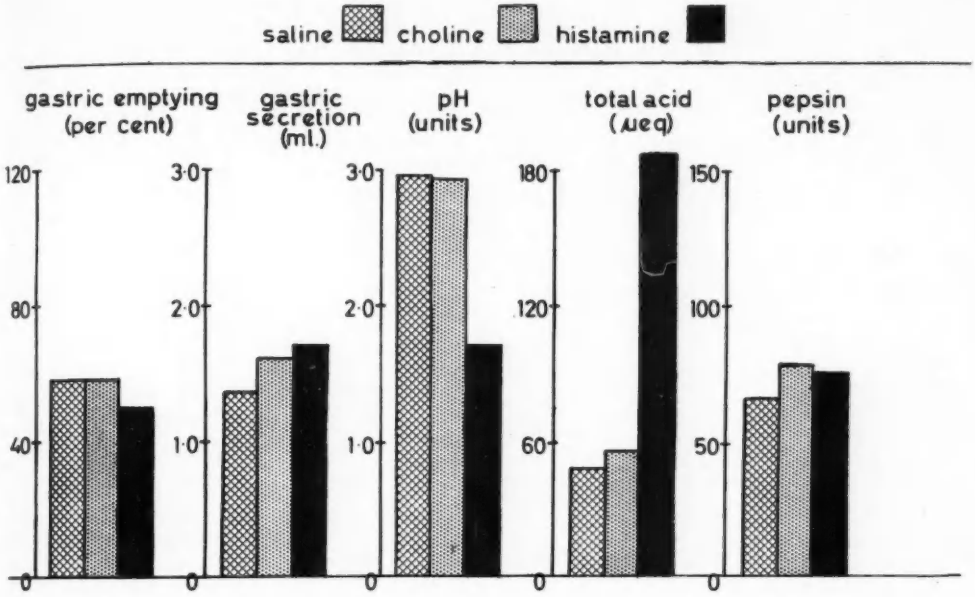

FIG. 3. *Effect of choline and histamine on gastric emptying (percentage of meal and secretion passed through pylorus), gastric secretion, pH, and total output of acid and pepsin.*

dose of choline chloride was equivalent, on an equimolar basis, to 278 mg./kg. body weight of histamine phosphate and it was within the range of dosage recommended by Komarov *et al.* (1944). Gastric analyses were also performed on 31 rats which received injections of histamine phosphate, 50 mg./kg. body weight.

The results are shown in Fig. 3. Choline chloride in a dose of 126·5 mg./kg. body weight was found to have no greater effect on gastric emptying, gastric secretion, acid production, or pepsin output than an equivalent volume of saline. These findings disagree with those of Komarov and his colleagues (1944) who found, in the anaesthetized rat with a ligated pylorus and a gastric fistula, that injections of choline chloride increased the volume of gastric secretion from 0·08 ml. to 0·33 ml./hour and increased the output of acid from 8·8 μEq. to 37 μEq./hour. These values are unusually low and even those of the choline-treated group are lower than the results obtained in the present study with injections of saline. In the light of subsequent improvements (Shay, Sun, and Gruenstein, 1954) that were made in the preparation of the 'pylorus-ligated rat' for the study of gastric function the technique used by Komarov *et al.* was crude and rather inadequate.

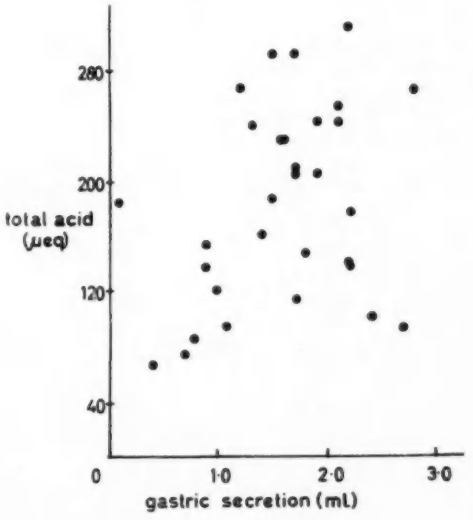

FIG. 4. *Relationship of total acid secretion to the volume of gastric secretion during the 45-minute test meal.*

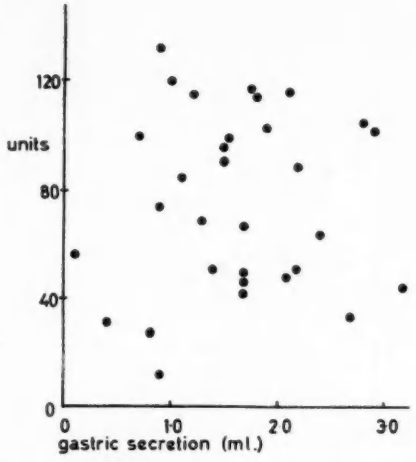

FIG. 5. *Relationship of total pepsin secretion to the volume of gastric secretion during the 45-minute test meal.*

It will be seen from Fig. 3 that although histamine phosphate had a pronounced effect on the output of acid its effect on pepsin secretion and gastric emptying was no greater than that of saline. The volume of gastric secretion was slightly greater in the histamine-treated group but the difference was not significant. However, when the individual results were plotted, there was a definite correlation between the amount of acid produced and the volume of gastric secretion which was significant at $P<0.01$ (Fig. 4).

No interrelationship was found between gastric emptying and the other indices. Of the other parameters, no correlation was observed between acid and pepsin secretion (Fig. 5). The finding of substantial amounts of pepsin in the gastric secretion of the saline-treated group and similar quantities in the histamine- and choline-treated groups suggests that pepsin is continuously secreted by the gastric mucosa. The failure of histamine to stimulate the secretion of pepsin in the rat is in accord with the findings in the dog and the cat (Linde, 1950). These results are in contrast to those found in man where histamine has been shown to be an effective stimulant of gastric pepsin secretion (Hirschowitz, London, and Pollard, 1957).

We are indebted to Mr. J. R. P. O'Brien and Mr. G. E. Newman for helpful advice and we are grateful to Mr. J. Halfacree, Miss Roberta Crawley, and Miss Cecily Bubb for technical assistance. This work was supported by a grant to Professor L. J. Witts from the Medical Research Council.

REFERENCES

Aitken, M. A., Spray, G. H., and Walters, G. (1954). *Clin. Sci.*, **13**, 119-126.
Anson, M. L. (1938). *J. gen. Physiol.*, **22**, 79-89.
Bowie, D. J., and Vineberg, A. M. (1935). *Quart. J. exp. Physiol.*, **25**, 247-257.
Friedman, M. H. F. (1943). *Proc. Soc. exp. Biol.* (*N.Y.*), **54**, 42-44.
Ghosh, M. N., and Schild, H. O. (1955). *J. Physiol.* (*Lond.*), **128**, 35P-36P.
Hanson, M. E., Grossman, M. I., and Ivy, A. C. (1948). *Amer. J. Physiol.*, **153**, 242-258.
Hirschowitz, B. I., London, J. L., and Pollard, H. M. (1957). *Gastroenterology*, **32**, 85-87.
Kay, A. W. (1953). *Brit. med. J.*, **2**, 77.
Komarov, S. A., Shay, H., Rayport, M., and Fels, S. S. (1944). *Gastroenterology*, **3**, 406-413.
Kyle, J., and Welbourn, R. B. (1956). *Ibid*, **30**, 593-597.
Lane, A., Ivy, A. C., and Ivy, E. K. (1957). *Amer. J. Physiol.*, **190**, 221-228.
Linde, S. (1950). *Acta physiol. scand.*, Suppl. 74.
Roe, J. H., and Dyer, H. M. (1939). *Proc. Soc. exp. Biol.* (*N.Y.*), **41**, 603-606.
Rose, B., and Browne, J. S. L. (1938). *Amer. J. Physiol.*, **124**, 412-420.
Shay, H., Sun, D. C. H., and Gruenstein, M. (1954). *Gastroenterology*, **26**, 906-913.
Thornton, G. H. M., and Clifton, J. A. (1959). *Amer. J. Physiol.*, **197**, 263-268.

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A re-valuation of Glass's method of fractional precipitation of gastric secretion

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SYNOPSIS The work of Glass has suggested that glandular mucoprotein and mucoproteose can be isolated by a fractional precipitation method from gastric secretion. These studies have failed to confirm this hypothesis and point out a fallacy of the original technique used.

Glass and Boyd (1948, 1949) published several papers describing in great detail a fractional precipitation method for estimating human gastric mucus secretion obtained on routine gastric aspiration (Fig. 1).

They claimed to have isolated from the dissolved mucin, two separate mucous substances:—1, Glandular mucoprotein, stated to be a homogeneous chemical compound closely associated with the intrinsic factor and secreted by a special group of cells, namely, the mucoid cells in the neck of the gastric glands situated in the body and fundus of the stomach (Glass and Boyd, 1949; Glass, 1953); 2, mucoproteose, a complex of intermediate products of digestion of gastric mucus (Glass and Boyd, 1949).

For the last 10 years Glass and his group have investigated the secretory patterns of glandular mucoprotein and mucoproteose in health and in patients with gastric lesions, and claimed to have obtained distinctive secretory patterns of diagnostic significance. These claims have been accepted by many other investigators, and have been quoted frequently in the medical literature dealing with gastric secretion (James, 1957; Kent and Whitehouse, 1955; Bettelheim-Jevons, 1958). The fractional precipitation method (Glass and Boyd, 1949) has been re-examined in our laboratory and the results indicate that the method was based on an erroneous assumption.

Glass and Boyd (1949) stated that pepsin is precipitated from the gastric secretion by the addition of ½ volume of 10% trichloracetic acid. We have been unable to substantiate this. We found (Fig. 1, step 1) that pepsin was not precipitated by trichloracetic acid but remained in the supernatant; it is, in fact, precipitated by acetone (step 2), which at the same time brings down the mucopolysaccharides. The whole of the pepsin in the precipitate is redissolved on adding 0·1N NaOH and is reprecipitated on acidification (step 3).

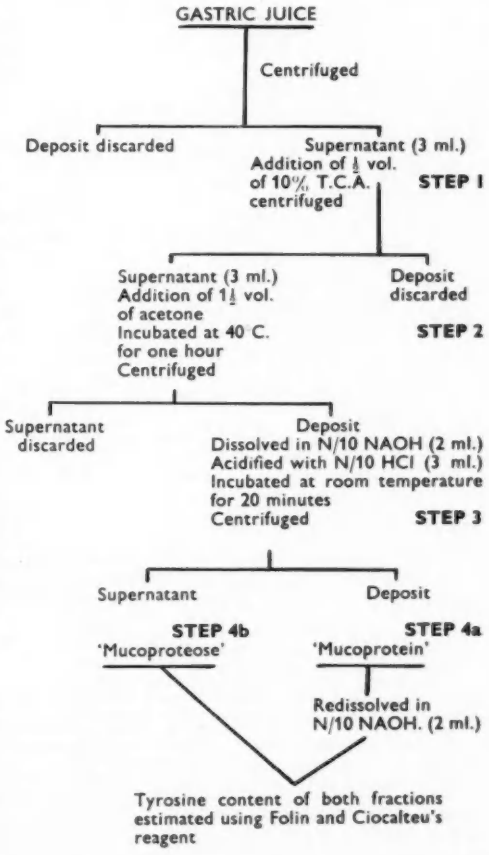
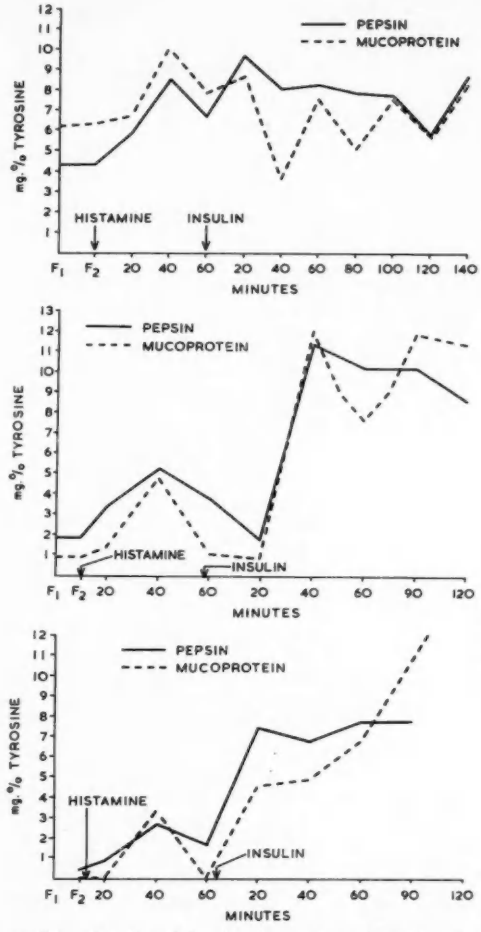


FIG. 1. *Diagram of the fractional precipitation method of Glass for the isolation of gastric mucoproteose and mucoprotein.*



FIGS. 2-4. *Results of fractional gastric analysis showing the glandular mucoprotein and pepsin content expressed as tyrosine.*

PROCEDURE AND RESULTS

The following investigations were carried out in order to determine the nature of the precipitated materials obtained from gastric secretions by fractional precipitation.

Fractional gastric aspirations were carried out using histamine and insulin as stimulants. The procedure was as follows:—

Two fasting specimens were aspirated at 10-minute intervals followed by intramuscular injection of histamine acid phosphate, 0·04 mg. per kg. body weight of the patient, followed by three aspirations at 20-minute intervals. The histamine injection was preceded by intramuscular injection of an antihistamine (mepyramine, 50 mg.). Intravenous insulin was then given, the dose depending on the fasting blood sugar (16 to 20 soluble units). Aspirations were carried out every 20 minutes; in two cases the last two specimens were aspirated at 30-minute intervals (Figs. 3 and 4). These fractional gastric aspirations were repeated on three patients. In each specimen the mucoprotein was estimated in terms of tyrosine, following Glass's procedure, and pepsin content was measured by its enzyme activity (Goodman's (1954) modification of West, Ellis, and Scott's (1952) method) using crystalline pepsin (Armour) as standard.

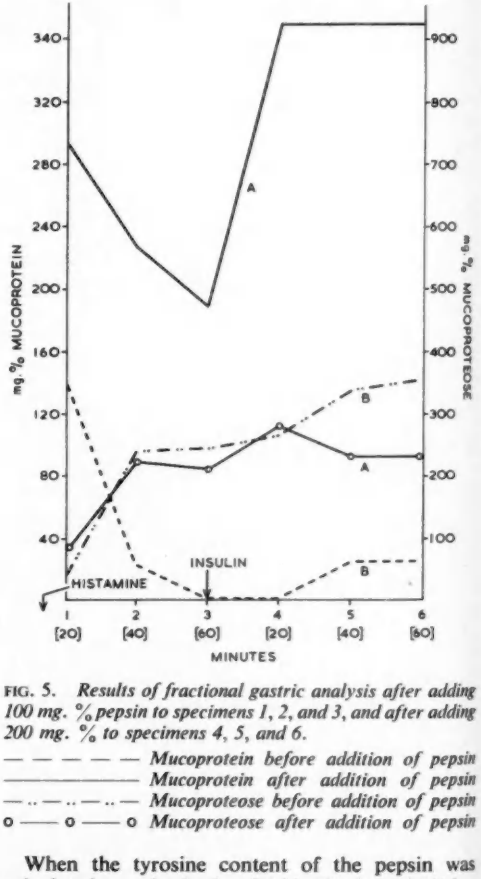


FIG. 5. *Results of fractional gastric analysis after adding 100 mg. % pepsin to specimens 1, 2, and 3, and after adding 200 mg. % to specimens 4, 5, and 6.*

— — — — — *Mucoprotein before addition of pepsin*
——————— *Mucoprotein after addition of pepsin*
— .. — .. — .. — *Mucoproteose before addition of pepsin*
o —— o —— o *Mucoproteose after addition of pepsin*

When the tyrosine content of the pepsin was calculated, on the basis of 9% (Block and Weiss, 1956), it was found to approximate to that derived from the 'glandular mucoprotein' (Figs. 2, 3, and 4). The gastric secretion had to be diluted 100-400 times and the findings multiplied accordingly. In

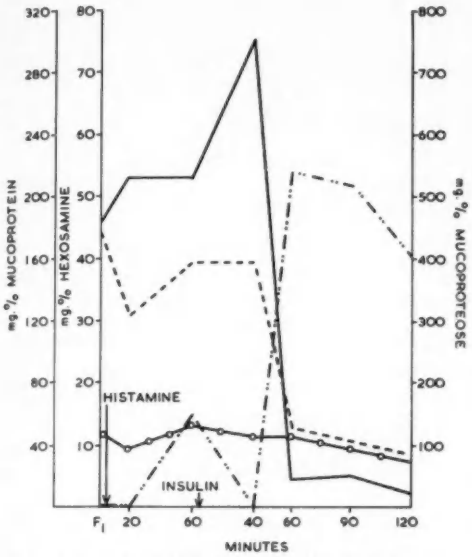


FIG. 6. *Results of fractional gastric analysis showing mucoprotein and mucoproteose and their hexosamine content.*
——————— *Mucoproteose*
— .. — .. — .. — *Mucoprotein*
o —— o —— o *Hexosamine with mucoprotein*
— — — — — *Hexosamine with mucoproteose*

spite of all these manipulations we find the two curves running so closely that we are forced to the conclusion that Glass's glandular mucoprotein is mainly pepsin assayed from tyrosine content.

Fractional gastric aspirations were performed following the procedure described above. Two aliquot portions (A and B) were taken from each specimen. Varying quantities of pepsin were added to the specimens of set A (Fig. 5). No pepsin was added to the specimens of set B. The glandular mucoprotein and mucoproteose were estimated in all the specimens of sets A and B.

The results show that the addition of pepsin increased the 'glandular mucoprotein', the increase being related to the amount of added pepsin. There was no increase in the mucoproteose.

This investigation appears to demonstrate conclusively that pepsin is the main source of the tyrosine used by Glass for estimating his 'glandular mucoprotein'.

Fractional gastric aspirations were performed as above. The glandular mucoprotein and mucoproteose were estimated as well as the hexosamine content of both these fractions. The results (Fig. 6) show no quantitative relationship between the glandular mucoprotein and the hexosamine content.

These data suggest that the precipitate does not consist of a single homogeneous and stoichiometric complex as would be expected of a pure mucoprotein. An investigation which is being carried on in our laboratory suggests that a sulphated mucopolysaccharide is precipitated together with a pepsin. The nature of this mucopolysaccharide is being studied. The larger portion of the hexosamine content of the gastric secretion was found in the supernatant (Fig. 1, step 4b). Here again no consistent quantitative relationship could be established between the tyrosine and the hexosamine content.

Our investigations suggest that the hexosamine is provided by a sulphated and non-sulphated mucopolysaccharide contained in the supernatant. Attempts are being made in this laboratory to isolate and study its substance.

CONCLUSION

Our findings do not support Glass's claim. The results warrant the conclusion that the tyrosine estimation used by Glass to calculate the 'mucoprotein' and 'mucoproteose' is a measure of the proteinous material only. The tyrosine of the 'mucoprotein' relates mainly to the pepsin content. Neither the 'mucoprotein' nor the 'mucoproteose' show even an approximately constant composition. It is suggested that this fractional precipitation scheme is not suitable for the isolation and determination of any mucoprotein or mucopolysaccharide secreted by the human gastric mucosa.

I wish to thank the Medical Research Sub-Committee of the Manchester Regional Hospital Board for a financial grant and encouragement. My thanks are also due to Mrs. M. Dodd and Mr. J. Howard for their technical assistance.

REFERENCES

Block, R. J., and Weiss, K. W. (1956). *Amino Acid Handbook—Methods and Results of Protein Analysis*. Thomas, Springfield, Illinois.

Bettelheim-Jevons, F. R. (1958). Protein-carbohydrate complexes. *Advanc. Protein Chem.*, **13**, 35-105.

Glass, G. B. Jerzy (1953). Hematopoietic activity of glandular mucroprotein from human gastric juice. *Gastroenterology*, **23**, 219-228.

——, and Boyd, L. J. (1948). Studies on dissolved mucin (mucoprotein) of the gastric juice. II. A new quantitative colorimetric method for the determination of total dissolved gastric mucin. *Rev. Gastroent.*, **15**, 511-519.

——, —— (1949). The three main components of the human gastric mucin: dissolved mucoproteose, dissolved mucoprotein, and mucoid of the gastric visible mucus. *Gastroenterology*, **12**, 821-878. (*Note* Pt 3 of article is on pp. 849-878.)

Goodman, R. D. (1954). *J. Lab. clin. Med.*, **40**, 872-879.

James, A. H. (1957). *The Physiology of Gastric Digestion* (Monogr. Physiol. Soc. No. 4). Arnold, London.

Kent, P. W., and Whitehouse, M. W. (1955). *Biochemistry of the Amino-sugars*. Butterworths, London.

West, P. M., Ellis, F. W., and Scott, B. L. (1952). A simplified method for determining the excretion rate of uropepsin. *J. Lab. clin. Med.*, **39**, 159-162.

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The effect of phenylbutazone (Butazolidin) on plasma pepsinogen activity

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SYNOPSIS Phenylbutazone in moderate dosage has been administered to 15 patients with a variety of musculo-skeletal disorders. A rise in plasma pepsinogen activity occurred in 13, generally reaching a peak in the first week of treatment; subsequently there was a fall in spite of maintaining a constant dosage. In four of six patients an anticholinergic drug failed to prevent a rise in plasma pepsinogen activity.

These findings are considered in relation to clinical observations on the time of appearance of gastrointestinal side-effects in patients receiving phenylbutazone.

The interpretation of the findings is discussed, and it is suggested that the rise in plasma pepsinogen activity induced by phenylbutazone may reflect either increased peptic activity in the stomach or a damaging effect of the drug on the stomach.

Phenylbutazone is a synthetic pyrazolone compound resembling aminopyrin in chemical structure. It is widely used in the treatment of arthritis and musculoskeletal disorders as it has analgesic and anti-inflammatory properties.

Among its several side-effects, those on the gastrointestinal tract are particularly common. Although stomatitis, constipation, and diarrhoea may occur, the most frequent complaints are of epigastric discomfort and nausea (Kuzell, Schaffarzick, Naugler, Gaudin, and Mankle, 1953). These symptoms may be associated with reactivation of peptic ulcers, and such lesions may be complicated by haemorrhage or perforation (Kirsner, 1957). Multiple superficial ulcers in the stomach have also been reported (Dilling, 1953). On the other hand, no lesion may be detectable in the stomach to account for the symptoms.

There have been few studies of the mechanisms by which phenylbutazone provokes epigastric discomfort and peptic ulceration. Kirsner and Ford (1955) showed that the secretion of acid rose significantly in 30 of 57 studies in 49 patients with single doses of 200 to 600 mg. Phenylbutazone intramuscularly in single doses of 50 to 400 mg. increased gastric acidity in 10 of 30 studies on 29 patients. These results have been confirmed by Bellomo and Fusco (1956). Neither the effect of repeated doses of phenylbutazone on gastric acidity in man nor its effect on peptic cell activity appears to have been investigated.

This investigation is concerned with the effect of repeated doses of phenylbutazone on plasma pepsinogen activity in man.

METHODS AND MATERIALS

Phenylbutazone was first given to nine women with various musculo-skeletal disorders (Table I). Patient 6 had received phenylbutazone three years previously; otherwise no patient had received the drug before. Initially, 400 mg. was given orally in two divided doses; in patients 1, 2, 3, 4, and 7 this was reduced to 100 mg. three times daily after the first two days. The patients

TABLE I

PATIENTS TREATED WITH PHENYLBUTAZONE

	Age in Years	*Diagnosis*	*Duration of Treatment in Days*
Patient 1 (L.W.)	19	Sacro-iliac strain	7
Patient 2 (M.M.)	44	Intervertebral disc lesion	21
Patient 3 (L.L.)	65	Osteoarthritis of hip	15
Patient 4 (H.S.)	18	Tenosynovitis of wrist	21
Patient 5 (Y.B.)	20	Postural backache	25
Patient 6 (A.M.)	50	Lumbar spondylosis	84
Patient 7 (J.McN.)	19	Postural backache	9
Patient 8 (J.M.)	18	Postural backache	7
Patient 9 (D.A.)	26	Postural backache	11

were instructed to take the tablets immediately after meals.

The duration of treatment varied. It was not considered justifiable to continue the use of a potentially dangerous drug when a discernible therapeutic effect was not produced in one to three weeks. The drug was stopped in patient 1 after one week because of an erythematous rash, after 11 days in patient 9 because the patient complained of epigastric discomfort, and after 84 days in patient 6 because of the development of oedema.

A second group of six patients was then studied (Table II). Each was given either 300 mg. or 400 mg. of phenylbutazone daily for one to two weeks, and in addition an anticholinergic drug. The anticholinergic drug used was oxyphenonium bromide (Antrenyl), 5 mg. being administered orally four times daily. These patients also received magnesium trisilicate powder, 4 ml. three times daily after meals.

TABLE II

PATIENTS TREATED WITH PHENYLBUTAZONE AND ANTRENYL

	Age in Years	*Sex*	*Diagnosis*	*Duration of Treatment (Days)*
Patient 10 (A.D.)	65	F	Osteoarthritis of hip	14
Patient 11 (E.M.)	60	F	Generalized osteo-arthritis	14
Patient 12 (F.H.)	74	M	Lumbar spondylosis	14
Patient 13 (T.B.)	30	F	Polyarthritis ? cause	7
Patient 14 (F.W.)	28	M	Reiter's syndrome	7
Patient 15 (E.T.)	66	F	Rheumatoid arthritis	14

In both groups plasma pepsinogen activity was measured twice before the drug was started and at intervals during treatment. Blood was taken at the same time on each day to avoid possible diurnal variations, and the plasma was stored at −15° C.

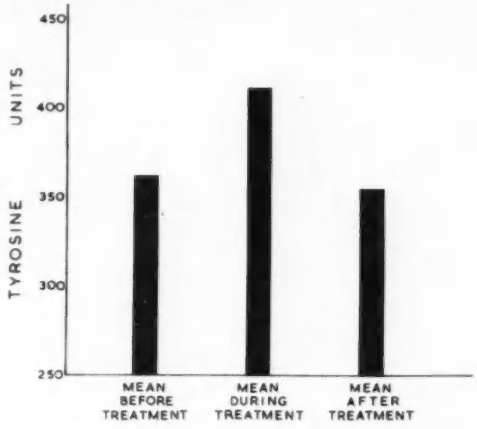

FIG. 1. *Average values for nine patients receiving phenylbutazone alone (baseline = 250 units).*

Mirsky's modification of Ansen's method of estimating tyrosine substances formed during enzymatic proteolysis was used (Mirsky, Futterman, Kaplan, and Broh-Kahn, 1952; Hoar and Browning, 1956). A series of standard solutions of tyrosine was included in each assay, as the Folin-Ciocalteau colour reaction is not constant. A Hilger absorptiometer as used by Nolan (1958) was used at a wave length of 430 μ. Results are expressed in tyrosine

TABLE III

PATIENTS TREATED WITH PHENYLBUTAZONE ALONE

Patient		*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*Mean for 9 Cases*
Before butazolidin	Day 1	400	320	480	290	320	300	290	310	570	
	Day 2	380	305	440	295	280	270	340	400	520	
Mean before		390	312·5	460	292·5	300	285	315	355	545	361·6
During butazolidin	Day 3	400	345		330	390	460			565	
	4			540					410		
	5	500	475		400		520			620	
	6			480		370		350			
	7	475	400		310		450			500	
	8			520		430		330			
	9		350								
	11									550	
	14		380		350	460	340				
	15			440							
	21		310		280	350	410				
	52						450				
	84						280				
Mean during		458	377	495	334	400	416	340	410	557	411·2
After butazolidin	1	355	300	—	300	290	380	260	390	550	
	2	350	320								
Mean after		352·5	310	—	300	290	380	260	390	550	354·5 (For 8 cases)

Plasma pepsinogen activity expressed as tyrosine units released per ml. of plasma.

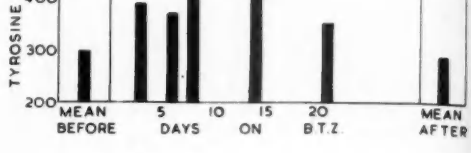

FIG. 2. *The effect of phenylbutazone on plasma pepsinogen activity in patients 2 and 4 (baseline = 200 units).*

FIG. 3. *The effect of phenylbutazone on plasma pepsinogen activity in patients 5 and 6 (baseline = 200 units).*

units, equivalent to microgrammes of tyrosine released per millilitre of plasma. The variation between duplicate estimations was found to be less than 7%.

RESULTS

The results in the first nine patients are shown in Table III and illustrated in Fig. 1. In all cases pepsinogen activity rose during treatment. The difference between the mean before and during treatment is highly significant ($P=0.001$). After treatment the values fell to pre-treatment levels.

Table III also shows that there was a tendency for the values to be highest during the first week of treatment. Thereafter they tended to fall towards pre-treatment levels in spite of a constant intake of phenylbutazone. The results in the four patients who were most completely studied over long periods are shown diagramatically in Figs. 2 and 3.

The results in the patients in the second group are shown in Table IV. Plasma pepsinogen activity rose in patients 10, 11, 13, and 15, despite the administration of an anticholinergic drug.

DISCUSSION

It has been recorded that epigastric discomfort and ulcer complications induced by phenylbutazone

TABLE IV

PATIENTS TREATED WITH PHENYLBUTAZONE AND ANTRENYL

Patient		*10*	*11*	*12*	*13*	*14*	*15*	*Mean for 6 Cases*
Before	Day 1	180	310	300	235	400	270	
butazolidin	Day 2	190	290	290	245	450	—	
Mean before		185	300	295	240	425	270	285·8
During	Day 3	190	350	220	250	460	330	
butazolidin	5	290	360	290	320	320	420	
	7	220	330	300	150	370	275	
	14	250	370	270	—	—	360	
Mean during		237·5	352·5	270	273·3	383·3	346·25	310·5

Plasma pepsinogen activity expressed as tyrosine units released per ml. of plasma.

occur most frequently early in the course of treatment (Kelly, 1954; Mauer, 1955). In one group of 35 patients with these side-effects, 23 developed symptoms in the first week (Freeland, Storey, and Thompson, 1953). Less commonly ulcer symptoms may develop after months of treatment. Reactions have also occurred after the drug has been withdrawn, and in this connexion it is interesting that phenylbutazone remains in the blood for 10 to 21 days after cessation of treatment (Bruck, Fearnley, Meanock, and Patley, 1954). The results recorded here, showing a peak rise in plasma pepsinogen activity during the first week of administration of phenylbutazone with a subsequent fall, may be relevant to the clinical observation of the time of greatest frequency of these gastrointestinal side-effects.

To draw more specific conclusions from these results depends on the interpretation of the relationship between levels of plasma pepsinogen activity and gastric function. The exact relation between peptic cell activity and plasma pepsinogen activity is uncertain. There is a virtual absence of pepsinogen activity in plasma and urine following total gastrectomy and in cases of pernicious anaemia (Chinn, 1953; Hirschowitz, 1955). Conversely, gastric pepsin secretion and plasma pepsinogen activity are both significantly higher in patients with duodenal ulcer than in normal persons (Chinn, 1953; Van Goidsenhoven, Wilkoff, and Kirsner, 1958). In addition, significant correlations have been shown between gastric pepsin and plasma pepsinogen activity in normal people by Janowitz, Levy, and Hollander, (1950) and Van Goidsenhoven *et al.* (1958).

Hirschowitz (1957) has stressed the constancy of plasma pepsinogen activity despite such stimuli as food, insulin, and anticholinergic drugs, all of which modify gastric pepsin secretion. He does not believe that the plasma activity gives a reliable indication of the secretory activity of the stomach. Goidsenhoven has, however, pointed out that the general pool of pepsinogen in the body may be quite large and it may therefore take some time for stimuli to affect the plasma activity. Thus, starvation for 24 to 48 hours and a similar duration of treatment with anticholinergic drugs is necessary to decrease plasma activity. The changes reported here may therefore reflect increased peptic cell activity.

An alternative explanation for our findings is suggested by an observation by Hirschowitz (1957). He found that plasma pepsinogen activity is increased by procedures which damage the gastric mucosa, such as destructive doses of histamine in dogs and guinea-pigs. He suggested that the rise in plasma pepsinogen in these circumstances was analagous to the rise in plasma of pancreatic enzymes following acute pancreatitis. It is possible, therefore, that the early rise in plasma pepsinogen activity which follows administration of phenylbutazone may reflect damage to the stomach rather than a change in secretory activity, and this possibility merits further study.

I am grateful to Dr. Sara Weiden for her help with the biochemical methods, to Dr. R. H. Melick for helpful criticism, and to Dr. K. J. Grice for permission to study patients under his care. I am also indebted to Professor R. R. H. Lovell for suggesting the investigation and for his encouragement.

REFERENCES

Bellomo and Fusco (1956). Foreign Letters; *J. Amer. med. Ass.*, **161**, 173.
Bruck, E., Fearnley, M. E., Meanock, I., and Patley, H. (1954). *Lancet*, **1**, 225-228.
Chinn, A. B. (1953). *Gastroenterology*, **25**, 14-23.
Dilling, N. V. (1953). *Lancet*, **1**, 1230-1231.
Freeland, D., Storey, G., and Thompson, N. (1953). *Ibid*, **1**, 1227-1229.
Hirschowitz, B. I. (1955). *J. Lab. clin. Med.*, **46**, 568-579.
—— (1957). *Physiol. Rev.*, **37**, 475-511.
Hoar, C. S. Jr., and Browning, J. R. (1956). *New Engl. J. Med.*, **255**, 153-158.
Janowitz, H. D., Levy, M., and Hollander, F. (1950). *Amer. J. med. Sci.*, **220**, 679-682.
Kelly, M. (1954). *Med. J. Aust.*, **2**, 504-507.
Kirsner, J. B. (1957). *Ann. intern. Med.*, **47**, 666-699.
——, and Ford, H. (1955). *Gastroenterology*, **29**, 1-17.
Kuzell, W. C., Schaffarzick, R. W., Naugler, W. E., Gaudin, G., and Mankle, E. A. (1953). *A.M.A. Arch. intern Med.*, **92**, 646-661.
Mauer, E. F. (1955). *New Engl. J. Med.*, **253**, 404-410.
Mirsky, A., Futterman, P., Kaplan, S., and Broh-Kahn, R. H. (1952). *J. Lab. clin. Med.*, **40**, 17-26.
Nolan, K. E. (1958). *Med. J. Aust.*, **2**, 831-833.
Van Goidsenhoven, G., Wilkoff, L., and Kirsner, J. B. (1958). *Gastroenterology*, **34**, 421-435.

Gut, 1961, 2, 44

Palliative surgery for gastric carcinoma

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SYNOPSIS This paper presents practical points concerning palliative surgery for carcinoma of the stomach. The results which may be obtained are evaluated.

Surgery for carcinoma of the stomach as we know it in Great Britain carries a high operative mortality and a low proportion of lasting cures. Most of the operations are therefore doomed to be no better than palliative. However, the likelihood that symptoms will be alleviated is very good, and palliation may reasonably be hoped to last for a year or two even though the patients are mostly elderly.

A palliative operation lengthens the lives of some and shortens those of others, but it relieves the vomiting in most and is without question potentially the most humane treatment. To deserve this description, however, it must avoid the implication that those who succumb shall have to suffer pointlessly in the interval between operation and death, intubated, splinted, dripped, and miserably uncomfortable. It is the doctor who has watched the sometimes disastrous sequelae of ill-planned, ill-managed, would-be surgical palliation who is apt to say, 'I would rather my patients died of their diseases than of their treatments'. The management described here avoids this threat.

A distant metastasis which is easily removable does not contraindicate palliative surgery. Indeed, the pathology is not always what it has been taken to be. A huge gastric ulcer in a patient of 77 was thought to be neoplastic, partly because there was a hard nodule at the umbilicus, but this turned out to be a sebaceous cyst. Another old lady who had a palliative total gastrectomy for a leather bottle stomach also had a mass of carcinomatous glands resected from the left axilla. These were found to contain spheroidal-celled growth, and a microscopic second primary was found in the breast at post-mortem examination after 14 months of excellent palliation.

PREPARATION FOR OPERATION

The preparation of patients thought probably to have incurable disease is not always meticulous. But, as bronchopneumonia is the commonest cause of post-operative death in these patients, oral preparation and dental preparation are particularly important and should not be omitted.

It is astonishing how often patients with neoplasms of the stomach, in spite of having spent a week or two in hospital, are found at operation to have stomachs packed with the shucks of haricot or broad beans and other coarse, undigested vegetable remains which must have made the accommodation and digestion of solid food impossible. A week of high-protein fluid feeding, sometimes preceded by a stomach wash, is therefore now used.

It is quite common for a patient with a carcinoma of the stomach to need resection of a piece of attached colon. All patients are therefore prepared for large-bowel surgery with a sulphonamide and neomycin. If the stomach is achlorhydric it is likely to be teeming with organisms, and this population as well as that in the colon is reduced by these drugs.

THE INCISION

Carcinomatous patients heal badly and are extra prone to infection and disruption of the wound, as well as to the peritonitis, ileus, and distension which often precede it. This has to be borne in mind when choosing the approach and its subsequent closure.

If there is any question that the proximal half of the stomach may be involved, a thoraco-abdominal exposure is used to allow of a more thorough and careful removal of involved tissue. Even for antral lesions this approach also allows a much more satisfactory dissection of the lymph nodes around the aorta, coeliac axis, and pancreas. The patient is put in the right lateral position and laparotomy is performed through a small left oblique abdominal incision, operability being assessed before the cut is continued into the chest.

THE LAPAROTOMY

If there is any hope of cure a standard radical operation is carried out, with either total, partial

proximal, or partial distal gastric resection as indicated by the age and fitness of the patient and the extent and position of the lesion.

If resection for cure is impracticable, the feasibility of palliative operation is considered. Short-circuiting operations — gastro-enterostomy, oesophago-jejunostomy, antral exclusion—have carried very high mortalities and morbidities and have been all too often disappointing in their results, though they are better than nothing when gastrectomy is out of the question. Gastric resection is far the most successful palliative operation where it is possible but it must not be thought that palliative gastrectomy is just a would-be-curative gastrectomy which has failed. When it is deliberately undertaken it is a different operation with different principles, though some of the details of management described may be adopted for all resections for gastric cancer with advantage. There is often some uncertainty whether resection is feasible and trial dissection may be necessary before any irrevocable step, such as division of the duodenum, is taken.

In borderline cases, if no symptoms urgently calling for palliation, such as vomiting or dysphagia, are as yet present, care is taken not to go too far before having to abandon the attempt to free the growth from vital structures. On the other hand, when unrelievable symptoms are so severe that failure to survive operation could only be counted a merciful release, a more determined effort is felt to be justified. In one such patient separation of an attachment left a gushing tear in the coeliac axis and aorta. The tear was repaired, however, and the the patient is still living, relieved of his pain and vomiting and doing his job.

Palliative resection is not considered justifiable if there are peritoneal seedlets and free fluid, or if carcinoma is invading the aorta, the coeliac or hepatic arteries, the superior mesenteric vessels, portal vein, or liver. However, intrahepatic metastases are not accepted to be a bar to palliative resection unless large or numerous, and involvement of spleen, pancreas, mesocolon, middle colic vessels, colon, diaphragm, or abdominal wall, are not held to preclude resection.

PALLIATIVE RESECTION

The principles of palliative resection are that it shall be as fully radical as required by the extent of the main growth but include no inessential prophylactic resection of uninvolved tissue. Glands are removed as may prove convenient, but even involved glands may be left if adherent to vital structures and separate from the main growth. They should not be cut across and dissection between them and the main mass has to be meticulous. There is no need to remove uninvolved omentum and the operation may be begun, as for benign ulcer, by opening through the great omentum into the lesser peritoneal sac. The mesocolon is often involved in growth and a patch of it may sometimes be cut away without difficulty. If the middle colic vessels are involved the mesocolon is opened and their origin identified. If this and the superior mesenteric vessels are clear the middle colic vessels are ligated, and the involved parts stripped up with the main mass and a piece of mesocolon. Pulsating colonic arteries are then sought on either side of the mass and the intervening segment of devascularized colon isolated between Ogilvie clamps, resected, and left attached to the stomach. Similar steps are taken if the colon itself is involved.

If there is any healthy stomach wall well clear of growth it is saved, and in this case an adequate blood supply to it must be assured by keeping intact either the trunk of the left gastric artery and its ascending branch or the splenic artery and some of its short gastric branches. It is my belief that the arteries of the oesophagus alone cannot be relied upon to nourish more than a tiny fringe of stomach in an elderly carcinomatous patient. If both splenic and left gastric arteries have to be sacrificed it is safer not to conserve more than the first three-quarters of an inch of stomach, though this much is kept if possible for the sake of its peritoneal covering. Sometimes, though the pancreas is involved, the splenic vessels may be dissected off it and saved for the sake of their short gastric branches. Several short pancreatic venous tributaries require ligation as well as one or two arterial branches.

An adherent body or tail of pancreas may be divided from its neck with ease after ligation or separation of the splenic vessels. If the splenic vessels are divided care is taken to recognize and, if possible, preserve the inferior mesenteric vein, though its retention is not vital unless transverse colon has been resected. Two oblique scalpel cuts are made, major 'bleeders' are picked up, and the pancreas then oversewn with fine silk with a crossing return stitch (Fig. 1).

If the neck and head of the pancreas are involved, an attempt is made to separate them from the underlying portal vein. If this proves practicable the operation is continued. As much pancreas as possible is preserved, but astonishingly little will maintain life. (This was shown by one of my patients who survived 11 months with a piece of pancreas no bigger than a shilling, then died suddenly in the street from haemorrhage from an intrahepatic haemangioma.) The hepatic artery is identified and uncovered before, and kept in view during, dissection

FIG. 1*a*.

FIG. 1*b*.

of the lesser curve lest it be inadvertently ligated. The gastroduodenal artery is not divided if this can be avoided.

It is considered particularly important that the anastomosis between stomach and duodenum or jejunum should be absolutely free from tension, as breakdown and leakage are more apt to occur in these subjects. For the same reason I prefer to use the jejunum rather than the duodenum with its unperitonealized friable posterior wall, and the interrupted silk or linen thread sutures of the seromuscular layer always take two bites each (figure-of-eight) and are not tied tightly.

When a substantial resection has included splenectomy an empty space appears below the left diaphragm which fills with small bowel. A rare complication which sometimes supervenes is volvulus of the bowel newly occupying this region, and most surgeons meet this sooner or later as a cause of death. Coils of jejunum are therefore deliberately arranged in the space to the best advantage possible at the end of the operation.

An important and valuable additional step is the construction of a temporary jejunostomy. This is potentially dangerous and liable to fatal intraperitoneal leakage unless made by a rigid, well-tested technique and with great care.

Two ordinary gastric suction tubes (or Jaques oesophageal tubes) are used and are introduced into the jejunum through openings, the first 3 or 4 in. below the gastro-jejunal anastomosis or the duodeno-jejunal flexure, and the other 2½ in. further down. The first tube, which should have large suction holes along 6 or 7 in. of its terminal length, is led back through the anastomosis, its tip being transfixed by a fine silk or thread suture and sewn to the greater curve of the stomach, taking a small bite. This stitch is loosely tied and buried by another which is figure-of-eight and picks up only the seromuscular coat. The second tube, which may be narrow, is passed 3 or 4 in. down the jejunum away from the anastomosis and is used for feeding. Both tubes are buried snugly in the wall of the jejunum Witzel-fashion, by a continuous fine silk suture, so that they emerge together. The tubes and the ends of both continuous silk sutures are passed through a single hole in a leaf of omentum and the sutures used to fasten the jejunum securely to the peritoneal edges of a small stab wound well separated from the main incision. The feeding tube is marked with a ligature outside the abdomen (Fig. 2).

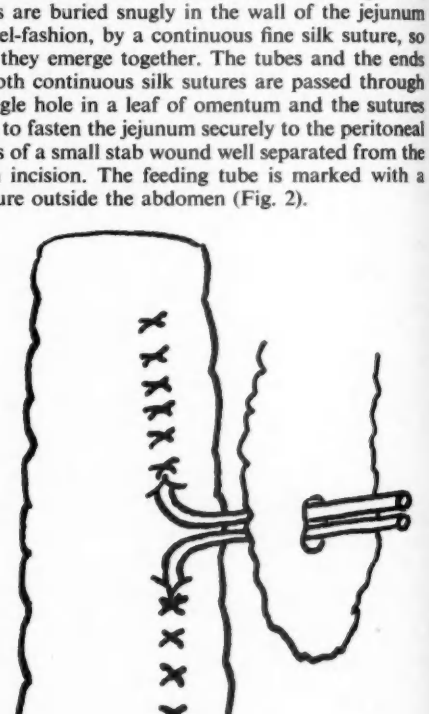

FIG. 2

Finally, a soft plastic drain, not a piece of hard corrugated rubber, is introduced down to the anastomosis and the stump of the pancreas if this has been partially resected. This drain is brought out through a separate stab wound in the flank.

Jejunostomy as an additional step in the technique of gastrectomy for cancer simplifies the post-operative management and is invaluable in the not infrequent cases of prolonged ileus or of leakage at the anastomosis. By dispensing with a nasal tube and often making possible the early discontinuation of intravenous infusion, it also ensures that those who do not recover after the operation shall suffer less discomfort during their last days.

The gastric tube is kept continuously sucked by a pump, at not more than 2 lb. to the square inch negative pressure, for the first three days or longer. If the patient's general condition is good he is allowed small frequent drinks of water from the second or third day provided they are seen to be immediately sucked out into the bottle of the continuous suction apparatus. Jejunal feeds are given freely when bowel sounds are present, but a cautious water or half-saline, half-milk drip may begin earlier if hourly aspirations of both tubes are performed. Unless jejunal feeds are small they are apt to cause colic and drip feeds are often tolerated best. If the discharge from the drain is copious and suggests leakage from the anastomosis, a methylene blue tablet is given by mouth which stains it immediately if a leak is present. If a leak occurs, oral feeds are stopped and continuous suction re-established until the leak heals. Jejunal feeds are continued, however, provided a methylene blue tablet in the jejunal feed does not stain the discharge.

PROXIMAL GASTRECTOMY

For a growth involving the cardia which is obstructing swallowing a thoraco-abdominal approach is always used. It is usually but not always necessary to divide the diaphragm but there should be no hesitation in resecting any small piece involved in the growth. If the lesion is too advanced for radical would-be curative resection, but a palliative resection is feasible, total gastrectomy is sometimes unnecessary and the distal part of the stomach may be conserved. The left gastric artery is divided at its origin and the spleen and most of the pancreas are resected. However, the blood supply to the gastric stump is adequate if the right gastric and the right gastro-epiploic arteries are left intact. The right gastric artery alone is not always enough and the epoploic arcade is deliberately and carefully preserved up to the level of resection.

Before the oesophago-gastric anastomosis is completed a gastric tube is passed just through the anastomosis into the oesophagus. The tube is tethered to the gastric wall by a silk stitch near the anastomosis and this stitch buried by another. The open end of the tube is then threaded onto a short curved length of metal rod or a gum elastic bougie to serve as a bodkin and guided down the duodenum and into the jejunum, where it is later delivered and used for suction as previously described.

After oesophago-gastric anastomosis a pyloroplasty is necessary as the vagi have been inevitably destroyed. My practice is to divide serosa and muscle only, in the line of the bowel, as in Ramstedt's pyloro-myotomy, and then sew up these coats transversely, as in the Mikulicz operation, with a single continuous suture.

If not even a palliative resection is possible an oesophageal dilator may be passed, and guided through the growth from within the abdomen. A plastic Mouseau-Barbin tube is then attached to its upper end and pulled into position in the stricture after the dilator has been delivered through a small opening in the stomach wall, or even the duodenal wall, distal to the growth. This technique may also be attempted in a case of non-resectable, leather-bottle stomach, instead of oesophago-jejunostomy with its high rate of failure.

AFTER-CARE

Survivors from very radical gastrectomies have difficulty in maintaining body weight and require very frequent small feeds. These should be mainly of protein and, unless bilous regurgitation is troublesome, should contain plenty of fat. Fruit and green vegetables should be almost completely avoided and extra vitamins will be required. Of minerals, probably only iron will need to be subsidized but two or three tablets daily of ferrous sulphate co. or its equivalent should be given. If the patient survives for more than a year or so regular injections of vitamin B_{12} may become necessary and a regular review of the blood picture is the rule.

Drinking with meals should be minimal, but water and fruit juice should be taken between meals, as well as milk drinks provided they do not precipitate the 'dumping' syndrome.

If much pancreas has been resected, steatorrhoea may be troublesome and pancreatin should be given as well as kaolin or chalk; the fat intake should be reduced. Insulin is not usually required even when only a tiny piece of pancreas has been left.

RESULTS

Over the years I have tended to undertake gastrectomy more and more often with the object of palliating or forestalling distressing symptoms, even in cases in which there was virtually no hope of cure. In this series, up to five years ago resection was performed in 59% of those who had undergone

TABLE I

SUMMARY OF OPERATIONS

Operations Performed	*All Operations*	*Laparotomy Only*	*Bypass*	*'Curative' Resection*	*Palliative Resection*	*All Resections*	*Resection Rate (% of All Operations)*
Over 5 years ago	41 (20, 49%)[1]	9 (7)	8 (7)	19 (5, 26%)	5 (1 20%)	24 (6, 25%)	59
Less than 5 years ago	69 (19, 28%)	9 (4)	7 (5)	25 (2, 8%)	28 (8, 29%)	53 (10, 19%)	77
Totals	110 (39, 35%)	18 (11)	15 (12)	44 (7, 16%)	33 (9, 27%)	77 (16, 21%)	70

[1]Deaths in parentheses.

laparotomy, but during the last five years this figure has risen to 77%. Nevertheless the overall operative mortality for patients who were explored surgically has been reduced from 49% to 28% over the same period, and the mortality for gastric resection has fallen from 25% to 19%. In the whole series over a half of those who died were over 70 years of age and an exactly similar number of septuagenarians survived resection.

At laparotomy patients were deemed beyond cure if the growth or the glands were adherent to an adjacent organ, or if there was an irremovable metastasis. Mortality for resection among such cases was 27%. Subsequent examination of surgical specimens has shown that even of the others very few in this series came to operation at a stage which offered reasonable hope of cure. Indeed, in all but three instances the serosal coat of the stomach had been breached by carcinoma, and lymph nodes were already involved. In those patients, thought at laparotomy to be worthy of an attempt at cure, resection cost the lives of 16% and, during the past five years of 8%.

Total resection had not been used unless the patient was relatively young and strong, or unless the extent of the lesion compelled it. The few undergoing total gastrectomy have therefore included a number with desperate and advanced lesions and half of them succumbed. The death rate for partial resections was 13%. Carcinoma of the cardia, often reported to carry the worst prognosis, has carried the best in this series. This may be partly due to the fact that, in the presence of dysphagia, operation has most often not awaited positive radiological findings. Another reason is that all have been operated upon by a thoraco-abdominal approach which gives a particularly good opportunity for clearing aerolar tissue and glands around the aorta, coeliac axis, and pancreas.

The five-year survival figures have been disappointing; but it must be borne in mind that the majority of the patients were elderly and their expectation of life was short even had they not had carcinoma. Indeed, the five-year survival figures which have been reported from some centres imply that the patients must have been a great deal younger than those commonly seen with carcinoma of the stomach in Great Britain. The results also reflect the advanced stage at which the patients have come to surgery.

Though patent medicine advertisements have led many of my patients to waste time trying a great variety of antacids before going to their doctors, it is regrettable that for many of them there has been further delay, sometimes too long, before they were referred to hospital for investigation. Even then some patients have wasted more valuable weeks because of the all-too-frequent attitude of 'a week

TABLE II

ALL RESECTIONS

x Years	*No. of All Operations x or More Years Ago*	*No. of Gastric Resections x or More Years Ago*	*No. of Patients Surviving Resection x or More Years Ago*	*No. of Patients Surviving x or More Years after Resection*	*Percentage of Surgical Patients Alive x or More Years after Operation*	*Percentage of Resection Patients Alive x or More Years after Operation*	*Percentage of Survivors from Resection Alive x or More Years after Operation*
½	103	72	57	46	45	64	81
1	95	66	52	37	39	56	71
1½	88	59	45	21	24	36	47
2	79	52	41	13	16	25	32
3	63	41	32	7	11	17	22
4	50	30	24	4	8	13	17
5	41	24	18	2	5	8	11

TABLE III

	x Years	*No. of Gastric Resections x or More Years Ago*	*No. of Patients Surviving Operation*	*No. of Patients Alive x Years or More after Operation*	*Percentage of Resection Patients*	*Percentage of Resection Survivors*
Palliative Resections	½	29	21	15	52	71
	1	26	19	12	47	63
	1½	23	16	3	13	19
	2	19	14	2	11	14
	3	12	9	0	0	0
	4	7	6	0	0	0
	5	5	4	0	0	0
Curative Resections	½	43	36	31	72	86
	1	40	33	25	62	76
	1½	36	29	19	53	66
	2	33	27	11	33	41
	3	29	23	7	24	30
	4	23	18	4	17	22
	5	19	14	2	11	14

or two more will not make any difference', and some patients have lost still more time because the results of their earliest investigations were negative. Among the latest 50 patients, seven had negative radiographs at the time of their first hospital consultation, and of eight cases of carcinoma of the cardia, there were negative radiographs in three, even at the time of operation. In one case a patient had undergone radiographic examination and oesophagoscopy for dysphagia at another hospital, with negative results, three months before being referred on account of persistent symptoms and weight loss; in another negative radiography and oesophagoscopy, followed by failure to get a view through a gastroscope, led to a delay of three months before laparotomy was decided upon. In a third patient gall-stones were seen in an otherwise normal barium meal radiograph. The patient was referred for these and at operation was found also to have a carcinoma at the cardia.

LONG-TERM RESULTS

In reading cure rates it is important to note whether these have been expressed as percentages of resections performed, of would-be-curative resections, or of survivors from resection. The proportion of patients undergoing resection also has a bearing on the results. In this series, of all the patients who underwent laparotomy (with or without resection) 44% were alive six months later; of those who were able to have resections 64%, and of those who survived resection, 81%. After two years the figures were 16, 25, and 32%. A patient who underwent a palliative gastrectomy had only a 50% chance of surviving operation and a subsequent period of life of six to 12 months, but of those who were readmitted after recurrence none was vomiting, unable to swallow, or in uncontrollable pain, and all died in coma. Twenty-two of the patients are alive at the present time, and this includes all of the only three

TABLE IV

	x Years	*No. of Gastric Resections Done x or More Years Ago*	*No. of Patients Surviving Operation*	*No. of Patients Alive x Years or More after Operation*	*Percentage of Resection Patients Alive at x Years*	*Percentage of Resection Survivors Alive at x Years*
Resections (all kinds) done over four years ago	½	30	24	17	53	71
	1	30	24	12	40	50
	1½	30	24	9	33	38
	2	30	24	7	23	29
	3	30	24	5	17	21
	4	30	24	4	13	16
	5	24	18	2	8	11
Resections (all kinds) done less than four years and more than six months ago	½	42	33	29	69	88
	1	36	28	23	64	82
	1½	29	21	12	41	57
	2	22	17	6	21	27
	3	11	8	2	18	25

patients whose lesions had not penetrated all coats of the gastric wall or reached lymph nodes.

The results have also been analysed separately for patients operated upon in recent years and those operated upon earlier (Table IV).

In spite of the policy of more frequent use of palliative resection, results are seen to have improved slightly, even from the point of view of long-term survival. Whether this is due to chance or experience it is impossible to say, for one or two extra survivals can make so much difference to percentage figures in a small series. The results for the earlier period cannot now improve, for all the patients are either dead or have already qualified as five-year survivals. In the later group, however, many are still living and the figures are accordingly improving all the time. There are therefore good grounds for hoping that these will ultimately achieve a more gratifying proportion of lasting cures.

SUMMARY

For carcinoma of the stomach which is beyond hope of cure palliative resection is the best treatment if it is feasible. The indications and contraindications are given. The operation has different principles from those of would-be-curative resection and calls for different techniques. However, some of these may be applied with advantage to all gastrectomies for cancer. They are described, and the principles of management and after-care are summarized. The results, both immediate and late, are given, and compared with those for operations hoped at the time to be curative.

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Framycetin sulphate (Soframycin) as a pre-operative bowel-sterilizing agent

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SYNOPSIS The author has investigated the bowel-sterilizing powers of framycetin sulphate, and has compared its potency with that of neomycin. He has found that the drug is well tolerated, and not toxic in doses which are sufficient to suppress faecal organisms satisfactorily.

In recent years the use of bowel-sterilizing agents has greatly facilitated gastrointestinal surgery, especially that of the large bowel. Given pre-operatively and combined with a course of bowel irrigation an operation field is obtained which is free from faecal contamination and where the faecal organisms are eradicated or greatly reduced in number. A number of chemotherapeutic agents and antibiotics have been tried. The sulphonamides and neomycin have been amongst those most widely used. The sulphonamides require a prolonged course of four or five days to be effective and are therefore not suitable where rapid sterilization is required. Neomycin is an effective agent for quick sterilization and good results may be obtained in 24 hours. The purpose of this paper is to report a clinical trial of a new sterilizing agent, framycetin sulphate (Soframycin), and to compare its effectiveness with that of neomycin.

Framycetin was isolated by Decaris in 1947. It is a broad-spectrum antibiotic affecting both Gram-positive and Gram-negative bacteria. It is both bactericidal and bacteriostatic. It has already been used as a local application in the treatment of infected wounds, skin infections, lung abscess, and in bladder irrigation. It has also been used to treat gastrointestinal infection and to prepare the bowel for operation. It is poorly absorbed when given by mouth and is not toxic. Large doses, however, produce nausea and vomiting. It should not be used parenterally as there is a danger of toxic action on the eighth cranial nerve and on the kidney.

METHOD

The following trial has been carried out on a wide variety of cases coming to intestinal surgery. Among the cases are included gut resections for carcinoma, diverticulitis, ulcerative colitis, Crohn's disease, closure of colostomy, and all but acute cases of intestinal obstruction. In many of these cases the bowel was also treated pre-operatively by lavage.

Three stool cultures were made in each case: (1) pre-operatively before administration of the sterilizing agent; (2) at operation from the transected bowel; and (3) post-operatively from the first stool passed.

Four different dosages of Soframycin were used, each dose being given to a group of 12 patients.

Group 1, 1½ g. twice daily for 24 hours, in a total dosage of 3 g. Group 2, 2 g. twice daily for 24 hours in a total dosage of 4 g. Group 3, 1½ g. twice daily for 48 hours in a total dosage of 6 g. Group 4, 1 g. hourly for four hours, then 1 g. four hourly for 20 hours in a total dosage of 9 g.

The results in these four groups were compared with those of another group of 25 patients who received neomycin pre-operatively. The dose of neomycin used was the same as for group 4 receiving Soframycin, *i.e.*, 1 g. hourly for four hours, then 1 g. four hourly for 20 hours. This dose of Neomycin is the standard used as a preoperative bowel sterilizing agent for some time now. At these dosage levels of Soframycin no toxic signs were met except in group 4 where a total of 9 g. was given in 24 hours. Nausea and vomiting occurred in over 60% of cases and the drug had to be stopped. With a similar dose of Neomycin nausea and vomiting occurred more rarely. With the lower doses of Soframycin, however, the drug is very well tolerated. Patients who at different times had received Soframycin and Neomycin often express a perference for Soframycin as it causes less discomfort from intestinal hurry.

RESULTS

Of the 12 patients in group 1 (Soframycin 3 g. in 24 hr.) five had no bacteria in the stool at operation. One of these stools contained yeasts. In the first post-operative stool bacteria were present in all but one patient.

In Group 2 (Soframycin 4 g. in 24 hr.) eight out of 12 patients had bacteria-free stools at operation. A further patient produced only a scanty growth.

In the first post-operative stool bacteria were present in all but three cases, one of these containing yeasts.

In group 3 (Soframycin 6 g. in 48 hr.) eight out of 12 patients had bacteria-free stools at operation, one of these containing yeasts. A further two patients produced only a scanty growth. In the first post-operative stool bacteria were present in all but two.

In group 4 (Soframycin 9 g. in 24 hr.) only four of the 12 patients tolerated the whole dose. The other eight patients vomited some of their tablets. Of these four, three had bacteria-free stools at operation. In the first post-operative stool bacteria were present in all but one.

Of the 25 patients on Neomycin (Neomycin 9 g. in 24 hr.), 17 produced a bacteria-free stool at operation. Of these five contained yeasts. A further two patients produced only a scanty growth. In the first post-operative stool seven patients were free from bacteria. Of these one contained yeasts. A further one produced only a scanty growth.

Further analysis of these figures is shown in Table I. It may be seen that Soframycin in dosages of 4 g. in 24 hr. or 6 g. in 48 hr. produces negative stool culture at operation in 66% of cases. This compares very closely with Neomycin where the figure is 68%. In a dosage of 3 g. Soframycin in 24 hr., however, the figure falls to 38%. At a dosage of 9 g. Soframycin in 24 hr. stool culture was negative at operation in 75% of patients who tolerated the drug. However, this cannot be regarded as a useful dose owing to vomiting, and the number of cases tolerating the drug is too small to be significant.

TABLE I

ANALYSIS OF RESULTS WITH SOFRAMYCIN AND NEOMYCIN

Soframycin (g.)	*Total Dose* (g.)	*No. of Cases*	*No. with Positive Stool Culture at Operation*	*No. with Negative Stool Culture at Operation*
1½, b.d., 24 hr.	3	12	7	5 (38%)
2, b. d., 24 hr.	4	12	4	8 (66%)
1½ b.d., 48 hr.	6	12	4	8 (66%)
1, hourly for 4 hr., then 1 4 hourly for 20 hr.	9	4 Test abandoned	1	3 (75%)
Neomycin 1 hourly for 4 hr. then 1 4 hourly for 20 hr.	9	25	8	17 (68%)

It was noted that apart from patients showing negative stool culture and scanty growth at operation, nearly all at all dosages showed a considerable reduction in the bacterial content of the stool. This was true both for Soframycin and Neomycin.

In those patients on Soframycin showing positive stool culture at operation, streptococci were found most often. *Ps. pyocyonea* was the next most frequent organism found, then coliforms, *Proteus*, *Cl. Welchii*, and *Staph. albus* in order of decreasing frequency.

In cases showing negative stool culture at operation yeasts occurred from time to time both with Soframycin and Neomycin.

DISCUSSION

From the results above it would appear that Soframycin is a useful bowel-sterilizing agent. It causes a marked reduction in the organisms in the stool, and when given in optimum dosage produces a sterile stool at operation in 66% of cases. The length of preoperative medication to produce these results is short and the drug is well tolerated except in very high dosage. Results compare very closely with those for Neomycin but dosages required are lower and patients do not complain of intestinal hurry. Dosage of 1½ g. twice daily for 48 hours or 2 g. twice daily for 24 hours produces good results. At no time were any toxic reactions noticed, but at higher doses some nausea and vomiting was produced.

SUMMARY

Framycetin sulphate (Soframycin) has been tried as a pre-operative bowel-sterilizing agent, and results have been compared with those for Neomycin.

Faecal organisms have been well suppressed at doses of 4 g. to 6 g. given over 24 to 48 hours.

The drug is well tolerated and is not toxic.

Very little intestinal hurry is produced.

Nausea and vomiting may occur at higher doses.

I should like to thank Mr. A. Lawrence Abel for help with this paper, members of the surgical staff of the Gordon Hospital on whose patients the trial was carried out, Dr. W. M. Edgar for his work on the bacteriology, and Roussel for supplying the Soframycin.

BIBLIOGRAPHY

Burrows, D. (1958). *Brit. med. J.*, **2**, 428-429.
Connell, J. F., and Rousselot, L. M. (1958). *Surgery*, **44**, 447-452.
Decaris, L. J. (1953). *Ann. pharm. franc.*, **11**, 44-46.
Fairbrother, R. W., and Williams, B. L. (1958). *Lancet*, **2**, 1353-1355.
Louwette, R., and Lambrechts, A. (1958). *Brit. med. J.*, **1**, 868-869.
Maccebe, A. F. (1959). *Practitioner*, **182**, 628-634.
Shidlovsky, B. A., Marmell, M., and Prigot, A. (1956). *Antibiotics Annual*, 1955-1956, p. 118-121.

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Pyogenic abscess of the liver

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SYNOPSIS The author records the case histories of 27 patients suffering from liver abscess admitted during the period 1946-60 to the Radcliffe Infirmary, Oxford. Seventeen were examples of multiple and 10 of single abscess. In most cases the primary source was determinable. Despite there being only one survivor of the 10 patients with a solitary abscess, the author considers the condition easily curable if recognized early.

In 1836, John Bright, physician to Guy's Hospital in London, published an account of eight patients with jaundice. Two died after short illnesses marked by rigors and jaundice, and the necropsy in each revealed multiple abscesses throughout the liver. Apart from emphasizing that neither patient had stones in the common bile duct, Bright did not speculate as to the cause of the abscesses. In retrospect, however, it seems reasonable to say that both patients had portal pyaemia, and although unrecognized as such they are the earliest description of the condition found by the present writer.

Ten years later, in 1846, Waller described a patient who died of acute appendicitis and at the necropsy was found to have had multiple abscesses of the liver with a purulent thrombophlebitis of the portal vein and its tributaries. Waller deduced correctly that the suppuration of the liver was a direct result of the inflamed appendix and to him must go the credit for recognizing portal pyaemia as a clinical entity.

By the turn of the century it was well known as a complication of appendicitis by the French (Achard, 1894; Feltz, 1895; Dieulafoy, 1898). In England, Guy's physicians and surgeons seem to have been particularly interested in the condition. Carrington (1883) reported nine cases, Bryant (1897) a further 11, and Taylor (1902) gave an excellent account of the clinical symptoms and signs with the pitfalls of diagnosis.

At the present time pyogenic abscess of the liver is an unusual disease and it is rare for any one clinician to see a large number of patients. The textbook triad of swinging temperature, rigors, and jaundice is more the exception than the rule so that the clinical picture is often confusing, the diagnosis difficult, and the treatment uncertain.

In this paper 27 patients with pyogenic abscess of the liver are considered. They were admitted to the Radcliffe Infirmary between 1946 and 1960. This 15-year span has been particularly chosen because at its beginning antibiotics first appeared and have been used increasingly ever since. Nine patients have been excluded in whom abscesses were due to ascending cholangitis from obstruction of the common bile duct and three where the abscesses were part of a general septicaemia. This has been done because neither of these two groups really falls within the province of the main argument.

SEX AND AGE INCIDENCE

There were 18 men (68%) and nine women (32%), and the average age of the 27 patients was 61 years. More relevant than these bare figures is the fact that nine of these 27 patients were in their 70s or 80s. It is, therefore, important to realize that nowadays liver abscesses are often an affliction of the old and feeble. In the past the average age has been considerably lower (Bryant, 1897; Rothenberg and Linder, 1934; Ochsner, DeBakey, and Murray, 1938) because such series had a predominance of cases of appendicitis which weighted the figures in favour of youth.

TYPE OF ABSCESS

Seventeen (64%) of the patients had multiple abscesses and 10 (36%) a single one. The right lobe was affected in 12 patients, the left in two, and both lobes in seven. Two patients died with multiple abscesses and two with large solitary ones, but the necropsy report is not clear about which part of the liver was affected. Two patients with appendicitis and the symptoms and signs of liver involvement recovered on antibiotics alone so that it is impossible to be sure of the situation of the abscesses.

CAUSE OF THE ABSCESS

The source of the primary infection is shown in Table I.

TABLE I

SOURCE OF PRIMARY INFECTION

	No. of Cases	*Diagnosis*
Group I Inflammation, ulceration, or perforation of the bowel	9 (35·7%)	Diverticulitis .....2 Perforation of peptic ulcer.....2 Perforation of simple ulcer of colon .....2 Leaking intestinal anastomosis..1 Necrotic growths of rectum....2
Group II Acute appendicitis	5 (21·3%)	
Group III Acute cholecystitis	2 (7·1%)	
Group IV Miscellaneous	3 (14·2%)	Total gastrectomy .....1 Multiple pancreatic abscesses....1 Abscess of femoral hernial sac..1
Group V No cause found	8 (21·4%)	

Amongst the early writers on portal pyaemia, acute appendicitis was by far the commonest cause. Bryant (1897) said that it was responsible in eight (40%) of the 20 patients recorded by himself and Carrington (1883) and Langdon Brown (1901) in a collected series of 64 cases found that 27 (43%) had appendicitis. The complication being considered something of an oddity, sporadic case reports continued to appear and by 1926 Eliason was able to find 53 scattered in the literature.

At present, appendicitis no longer plays such a large part in the aetiology of liver abscesses. Ochsner *et al.* (1938) found that it only accounted for 10·6% of their personal cases and Bourne (1954) quotes a figure of 18%; in the present series of 27 patients there were five with appendicitis, an incidence of 18%. In so far as there is no case selection at the Radcliffe Infirmary and no 'emergency' is ever refused admission, it is fair to say that nowadays in just under a fifth of patients with liver abscesses, appendicitis is the cause. Over the same 15-year period, 8,638 patients were admitted with acute appendicitis so that portal pyaemia occurred as a complication in 0·05%.

The most interesting group are the eight patients who had no obvious primary focus of infection. Traditionally, such abscesses have been given the rather ungainly title of 'cryptogenic'.

There is little doubt that the single liver abscess is much more likely to appear *de novo* than the multiple. In the present series six out of 10 single abscesses had no obvious cause whereas this only happened twice in the 17 patients with multiple abscesses. This is confirmed by other writers. Rothenberg and Linder (1934) reported that 19 out of 24 solitary abscesses were cryptogenic and McFadzean, Chang, and Wong (1953) found the same in 12 of their 14 patients. Bourne (1954) says that twice as many cryptogenic abscesses are single as multiple.

Even accepting the fact that there is no obvious source of infection within the portal area, it is still plain that the abscess is not a primary event. Lepehne (1929) has argued that dissemination of septic emboli by the hepatic artery may account for some cases of multiple abscesses and Rothenberg and Linder (1934) have taken much the same view with the single abscess. These last two authors compare the solitary liver abscess to osteomyelitis or a carbuncle of the kidney and in support of their views claim that nine of their 24 patients had evidence of focal infection.

Beaver (1931) denied that infection could reach the liver by the hepatic artery except in the course of a generalized septicaemia. He went on to maintain that in all liver abscesses, single or multiple, there must be a primary focus somewhere within the portal area. This may be relatively so slight in character that its presence is overshadowed by the secondary abscess in the liver.

It is impossible to decide definitely between these two theories although the second would seem to be the more plausible of the two. Nevertheless, in fairness it has to be admitted that in the six fatal cases of cryptogenic abscess in this series a careful necropsy failed to reveal any source of infection within the abdomen or elsewhere in the body. Of the two patients with no cause for multiple abscesses one recovered (Case 1) and the other died (Case 2). At necropsy in this second patient there was pus in all the veins draining the sigmoid colon but the bowel itself seemed normal. These findings would seem to lend support to Beaver, as it is hard to believe that the infection could have come from anywhere but the gut.

DIAGNOSIS

'The chief aid to the diagnosis of the disease is the knowledge that it may occur' (Taylor, 1902). Although it is impossible to say that any one symptom or sign occurs in every case, some appear sufficiently often that when considered together they form a reasonably coherent clinical picture. The chief symptoms and signs are shown in Fig. 1, and the incidence compared with the personal series of 47 cases of Ochsner *et al.* (1938).

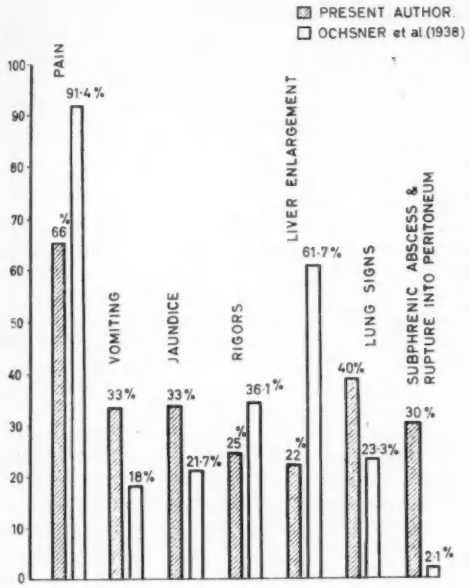

FIG. 1. *Diagram of signs and symptoms in present series compared with those found in the series of Ochsner* et al. (1938).

PAIN AND VOMITING Pain in the epigastrium and under the right costal margin was the most constant symptom, being present in two-thirds of the patients. In contrast vomiting is unusual and was absent in nearly two-thirds. The divorce between these two common symptoms is peculiar to liver abscesses and helps in distinguishing them from most other upper abdominal conditions in which pain and vomiting usually go hand in hand.

JAUNDICE Jaundice is usually considered a *sine qua non* of diagnosis but in fact it was only found in a third of the patients. It was present in about half those with multiple abscesses but in only one of the 10 with a single abscess. Jaundice seems particularly liable to appear when the appendix is at fault. All five patients with appendicitis in the present series were jaundiced, and Eliason (1926) says that it is invariably present when the abscess, whether single or multiple, complicates appendicitis. As this condition no longer monopolizes the aetiology, jaundice has become rarer.

In two patients of the present series, however, it was the only evidence of liver involvement. As the two histories are virtually identical only one is given (Case 3).

It might be argued that infection of the liver was not the cause of the jaundice but it is difficult to see how else it could be explained. Presumably the antibiotics in each patient successfully aborted the attack before extensive suppuration had taken place.

TEMPERATURE AND RIGORS Nearly all the patients had some degree of pyrexia but only a quarter the traditional 'swinging' septic chart, which was twice as common in those with multiple abscesses. The majority merely confirmed Bryant's point, made in 1897, that any type of temperature can occur.

As might be expected from this, rigors were exceptional and were only recorded in a quarter of the patients, of whom the overwhelming majority had multiple abscesses.

ENLARGEMENT OF THE LIVER Although a palpable tender liver is mentioned by most writers, it was found in less than a quarter of the present series. This figure is probably too low, because Ochsner *et al.* (1938) found enlargement in nearly two-thirds of their cases and both Rothenberg and Linder (1934) and McFadzean *et al.* (1953) say that the liver could be felt in nearly all their patients with a solitary abscess. It should be remembered, however, that as the increase in size is sometimes upwards, it may only be detected on a radiograph showing elevation of the right diaphragm.

Eliason (1926) mentions oedema and tenderness of the tissues over the right lower ribs as two valuable and constant signs but neither was detected in any of the patients in this series. This may be an error of clinical omission rather than commission.

SIGNS AT THE BASE OF THE RIGHT LUNG John Bright in the account of his second patient mentions that there was dullness at the base of the right lung. Most writers since then have made the same observation, which, if correctly interpreted, may be of considerable diagnostic value. It may equally prove a snare by distracting the attention of the clinician from the abdomen to the chest. Eliason (1926), in 53 cases of liver abscess collected from the literature, found that the initial clinical diagnosis in most had been a right basal pneumonia.

As the lung infection is in part due to the enlarged liver pressing upwards and causing a basal collapse, it is more commonly present with the single abscess. In the present series half of the patients with a solitary abscess had lung signs whereas these were only found in a third of the multiple abscess cases.

SUBPHRENIC ABSCESS AND RUPTURE INTO THE PERITONEUM If the abscess continues to grow it may finally burst into one of the subphrenic spaces or

else into the general peritoneal cavity. This happened in just under a third of the present patients, five developing subphrenic abscesses and three dying of general peritonitis. Both single and multiple abscesses seem equally liable to these complications.

The development of a subphrenic abscess (Case 4) may further confuse the diagnosis by masking the underlying liver infection. Granger (1930) has drawn attention to radiological differences in the chest films which may help in distinguishing between a 'simple subphrenic' and one secondary to a liver abscess. In the former the costophrenic angle is obliterated in the postero-anterior view of the chest and the posterior costophrenic angle on the lateral view. On the other hand, where there are both liver and subphrenic abscesses present, it is the cardiophrenic angle (instead of the costophrenic) which disappears on the postero-anterior view and the anterior (not the posterior) costophrenic angle is absent on the lateral view.

RADIOLOGY

Radiography may also help in the diagnosis of a liver abscess in one of three ways: (1) by showing elevation and fixation of the right half of the diaphragm together with changes in the overlying lung field (Pancoast, 1926); (2) an abscess of the right lobe may produce distortion of the right renal pyelogram (Yates, 1953); (3) an abscess in the left lobe may deform the lesser curve of the stomach in barium meal examination (Miles, 1936).

LABORATORY INVESTIGATIONS

The white cell count tended as a rule to be raised, being higher in the patient with multiple than single abscess. There were, however, many inconsistencies between the condition of the liver and the degree of leucocytosis, comparatively low counts sometimes going with extensive suppuration.

One valuable investigation is the estimation of the serum alkaline phosphatase. Hirschowitz (1952) says that if this is raised and all the other liver function tests are normal, it is strongly suggestive of either an abscess or a growth within the liver.

DISCUSSION

In the late nineteenth century physicians were only reluctantly allowing typhilitis to fade into a historical limbo and appendicitis to replace it on the surgical scene. The result was that many patients died of advanced peritonitis with their livers riddled with abscesses. The clinical picture of these unfortunates, drawn by the English and French writers of that time, has now become part of surgical lore. Case 5 could equally find a place in the modern textbook or the late Victorian surgical journal. Yet of the 17 patients with multiple abscesses in the present series it was only the five with appendicitis who in any way conformed to this classical pattern. The rest were exceptions to the rule.

Two patients (Cases 6 and 7) with diverticulitis and multiple abscesses had no clinical evidence of colonic inflammation so that their rigors and jaundice were misinterpreted as due to stones in the common bile duct. Four similar cases with a 'silent' diverticulitis causing liver abscesses were reported by Shaldon (1958). A third patient (Case 1) had such severe upper abdominal pain that he was diagnosed as a perforated peptic ulcer. In these three patients a primary focus of infection was either inconspicuous or absent so that the liver abscesses were misdiagnosed as two more common upper abdominal conditions. In two of the three (Cases 1 and 7) the correct diagnosis was made at laparotomy and at necropsy in the third (Case 6).

Amongst a further seven patients, four had general peritonitis, two late necrotic rectal growths, and the last a pancreas full of abscesses. Some were moribund on admission and all were dead within 48 hours. In this group the liver abscesses were silent in so far as any local symptoms or signs were swamped by the severity of the primary lesion. Diagnosis if not impossible was certainly academic.

The last two patients do not fit into any well-defined group. One (Case 4) has already been discussed. The other developed what was thought to be a subphrenic abscess after a total gastrectomy. He soon died and the necropsy showed extensive suppuration throughout the portal tracts. The source of the infection was probably the duodenal stump. This patient was never jaundiced and it was this that mislead the clinicians. It is therefore worth re-emphasizing that jaundice is rare in liver abscesses (other than those which complicate appendicitis) and in fact was only found in three of the 11 patients who died with multiple abscesses.

On the whole the solitary abscess is less fickle in its clinical presentation than the multiple. Yet in three patients of the present series the diagnosis was well nigh impossible. Two of the three were admitted and died in diabetic coma and the third had a general peritonitis from rupture of the abscess. A fourth patient had a laparotomy, when the abscess was found and successfully drained. In the remaining six the abscess was unsuspected and therefore undiagnosed until necropsy. Stokes (1960) has suggested that persistent upper abdominal pain with an unexplained fever should be sufficient to arouse suspicion of a solitary abscess. This may be true

but the most important single step in diagnosis is to remember that the condition exists. If this is done the answer to the clinical riddle is not difficult.

TREATMENT

The mortality figures for the series are shown in Table II.

TABLE II

MORTALITY IN THE SERIES

	Number of Patients	*Survivors*	*Mortality (%)*
Multiple abscesses	17	6	66
Solitary abscesses	10	1	90
Whole series	27	7	75

Pyogenic abscess of the liver has always been considered a peculiarly lethal affliction. Dieulafoy (1898) believed that death was inevitable and he emphasized that this high mortality was one of the prices to be paid for failing to diagnose acute appendicitis. This fatalistic view was confirmed by the 100% mortality of the Guy's Hospital series (Carrington, 1883; Bryant, 1897). It is true that even in these early days there was the occasional survivor after drainage of the abscess (Treves, 1894; Morton, 1897; Loisson, 1900), but these were isolated triumphs.

Forty years later the prospect had only improved slightly. Ochsner *et al.* (1938) found a mortality of 72·3% in their personal series and 79·6% in a collected series. Without operation, there was a 100% mortality in both groups. As can be seen from these figures in surgical drainage lay the only hope of survival. This met with some success in the case of the single abscess but all too often the multiple abscesses are so widely and deeply scattered that it is ineffective.

The appearance of antibiotics has considerably improved although not entirely transformed the outlook. Shaldon (1958) has insisted that antibiotics in themselves are not enough but must always be combined with drainage. The present writer feels that this is too sweeping a statement, because four of the five patients in this series in whom the liver infection followed on appendicitis recovered on antibiotics alone. The fifth was explored and a small collection of pus found over the right lobe of the liver which was softened over an area of 8 cm. in diameter. A drain was put down to this point but it is dubious whether it played much part in the patient's recovery. The sixth and last survivor (Case 1) in the multiple abscess group did have his abscesses drained.

Corry (1960) believes that in severe cases the antibiotics must be given intensively and continuously over a long period if the liver infection is finally to be brought under control. The truth of this teaching is borne out by the fact that in three of the patients of the present series the chemotherapy had to be prolonged for two months before recovery was finally assured. The necessity for this lengthy treatment was not realized in one patient (Case 7) who eventually died. The present writer played a considerable part in the treatment of this man and in retrospect I feel that we gave too little chemotherapy for too short a time. The result was that as soon as he left hospital the infection within the liver became active again and he died.

The solitary abscess has always provided a more hopeful prognosis than the multiple. Twenty-five years ago, Rothenberg and Linder (1934) had 14 survivors amongst 24 patients. In more recent years McFadzean *et al.* (1953) successfully treated 14 patients by simple, closed aspiration and antibiotics, and Stokes (1960) reported three patients who recovered after open drainage and systemic antibiotics. The one survivor of the 10 patients with a solitary abscess in the present series also recovered after the pus was drained.

An early diagnosis is the heart of the matter in the treatment of liver abscesses. Yet, it must be admitted that of the 11 patients who died with multiple abscesses, seven had such extensive intra-abdominal suppuration that death was almost certain. The mortality in this condition will probably always be high because the primary focus is so often lethal in its own right. This does not apply to the solitary abscess and it was a failure of diagnosis in the present series that was responsible for the mortality of 90%. This failure in turn was due to the fact that a solitary liver abscess was never considered as a possibility so that many of these patients died by default with an easily curable condition.

CASE REPORTS

CASE 1 F.D. (R.I.253392), a man aged 54 years, was admitted on 11 March 1958 with acute upper abdominal pain. He had a rigid upper abdomen, and clinically was diagnosed as a perforated peptic ulcer. At operation, there was a multiloculated abscess 5 cm. in diameter in the right lobe of the liver and a similar but slightly smaller one in the left lobe. There was no obvious primary focus of infection. The abscesses were drained and a course of terramycin was given post-operatively. The patient made an uneventful recovery, and left hospital a fortnight after his admission. A swab of the pus grew *B.coli*.

CASE 2 B.H. (R.I.100395/49), a man aged 31 years, was admitted on 9 January 1949 with what was thought to

be acute appendicitis. At operation, however, a normal appendix was removed. He left hospital but continued to complain of vague abdominal pain for which no cause could be found. About a week before his death he was admitted to the local cottage hospital where he was found to have a distended, tender abdomen. His condition steadily deteriorated and he died on 11 April 1949. At necropsy the peritoneal cavity contained 2½ litres of pus which came from a ruptured abscess 10 cm. in diameter in the left lobe of the liver, which also contained more abscesses. All the veins draining the sigmoid colon were full of pus which spread up into the splenic and portal veins as well. Although the pelvic colon was thickened it contained no lesion to account for the pylephlebitis.

CASE 3 W.S. (R.I.232136), a man aged 73 years, was admitted on 4 January 1960 with acute appendicitis. At operation a perforated gangrenous appendix was found and removed.

Postoperatively he started a course of terramycin but on the second day became jaundiced with tenderness in the right hypochondrium although the liver was not palpable. He had no rigors and only ran a low-grade fever. Penicillin was added to the terramycin and over the course of a week the jaundice slowly faded. The antibiotics were stopped on the eleventh post-operative day and the patient left hospital two weeks after the first appearance of the jaundice.

CASE 4 S.L. (R.I.55785/46), a man aged 72 years, was admitted on 4 April 1946 with the symptoms and signs of a perforated peptic ulcer. A straight radiograph of the abdomen showed a large amount of free air under the right diaphragm. He was treated conservatively with gastric suction, intravenous fluids, and a course of sulphadiazine. His course was stormy and by 26 April it was clear that he had a right subphrenic abscess. This was drained through the bed of the eleventh rib but the patient died 10 days later.

At necropsy there were multiple abscesses in the right lobe of the liver, one of which had ruptured through the bare area to form the subphrenic collection.

CASE 5 M.S. (R.I.265974), a man aged 17 years, was admitted on 9 December 1958 with a history of 14 days' abdominal pain, seven days' rigors, and five days' jaundice. A clinical diagnosis of a general peritonitis from a perforated appendix and portal pyaemia was confirmed at operation. The liver was enlarged and reddish-purple but had no obvious abscesses on its surface. Over the next two months he was given courses of penicillin and streptomycin, penicillin and sulphadiazine, terramycin and aureomycin, one following the other without pause. The jaundice rapidly faded but he continued to run a swinging temperature with rigors for many weeks. He finally left hospital, symptom free, nine weeks after his admission.

CASE 6 J.R. (R.I.119499), a man aged 69 years, was admitted on 5 February 1950. For five days he had been having right-sided abdominal pain with rigors and he became jaundiced shortly after entering hospital. He was thought to have stones in the common bile duct but in spite of a course of penicillin and sulphadiazine, the jaundice steadily deepened. His condition was too poor to permit a laparotomy and he finally died on 23 February. At the necropsy there were multiple abscesses in the right lobe of the liver and an acute diverticulitis of the sigmoid colon.

CASE 7 S.G. (R.I.233487), a man aged 59 years, was admitted on 10 December 1956 with a history of a fortnight's rigors and jaundice. He was clinically thought to have stones in the common bile duct. At laparotomy, however, he was found to have an acute diverticulitis of the sigmoid colon and the liver was enlarged and friable although there were no obvious abscesses. He was started on a course of antibiotics, and a month after the operation the right lobe of the liver was needled and pus aspirated which grew *B.coli* on culture. The antibiotics were continued and his clinical condition improved, the rigors ceased, and his temperature returned to normal. He left hospital eight weeks after the operation but relapsed at home and died three weeks later. No necropsy was done.

SUMMARY

Twenty-seven patients with pyogenic abscesses of the liver are reviewed. Seventeen patients had multiple abscesses and 10 a solitary abscess. A third of the patients were in their seventh or eighth decade.

Multiple abscesses usually have an obvious cause but six out of 10 solitary abscesses were cryptogenic. Appendicitis at present accounts for about a fifth of the cases of portal pyaemia.

Upper abdominal pain is the commonest symptom. Jaundice occurred in only a third of the patients and rigors in a quarter.

Antibiotics can be very valuable in treatment but in severe cases they must be given intensively and for a prolonged period. Drainage is not always necessary.

There was a mortality of 66% in the multiple abscess group and 90% in those with a solitary abscess. The single abscess is easily curable if diagnosed and it was failure of diagnosis that was responsible for the high mortality in this group.

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REFERENCES

Achard, C. (1894). Infection due foie compliquant l'appendicite: pathogénie des abcès aréolaires. *Bull. Soc. méd. Hôp. Paris*, 11, (3rd ser.), 793-805.

Beaver, D. C. (1931). Granulomatous abscess of the liver of pyogenic origin. *Amer. J. Path.*, **7**, 259-276.

Bourne, W. A. (1954). The diagnosis of pyogenic abscess of the liver. *Lancet*, **2**, 1093-1094.

Bright, J. (1836). Observations on Jaundice; more particularly on that form of the disease which accompanies the diffused inflammation of the substance of the liver. *Guy's Hosp. Rep.*, **1**, 630-637.

Brown, W. L. (1901). Suppurative Pylephlebitis. *St Bart's Hosp. Rep.*, **37**, 95-135.

Bryant, J. H. (1897). Suppurative Pylephlebitis. *Guy's Hosp. Rep.*, **54**, 77-112.

Carrington, R. E. (1883). Cases of multiple small abscess of the liver. *Ibid*, **26**, 374-438 (n.s. **4**).

Corry, D. C. (1960). Personal communication.

Dieulafoy (1898). La foie appendiculaire; abcès du foie consécutifs à l'appendicite. *Sem. méd.* (*Paris*), **18**, 449-452.

Eliason, E. L. (1926). Pylephlebitis and liver abscess following appendicitis. *Surg. Gynec. Obstet*, **42**, 510-522.

Feltz (1895). Appendicite compliqueé d'abcès aréolaire du foie. *Bull. Soc. méd. Hôp. Paris*, **12** (3rd series), 321-326.

Granger, A (1930). Radiological signs of subdiaphragmatic abscess. *New Orleans med. surg. J.*, **82**, 748-751.

Hirschowitz, B. I. (1952). Pyogenic liver abscess; review with case report of solitary abscess caused by *Salmonella enteritidis*. *Gastroenterology*, **21**, 291-299.

Lepehne, G. (1929). Die Erkranbugen der Leber und Gallenwege der Leberabszess. *Münch. med. Wschr.*, **76**, 849-851.

Loisson, E. (1900). Des Suppurations intra et péri-hépatiques d'origine typhlo-appendiculaire. *Rev. Chir.*, **21**, 522-539.

McFadzean, A. J. S., Chang, W. P. S., and Wong, C. C. (1953). Solitary pyogenic abscess of liver treated by closed aspiration and antibiotics; report of 14 consecutive cases with recovery. *Brit. J. Surg.*, **41**, 141-152.

Miles, J. M. (1936). Roentgenological diagnosis of abscess on concave surface of liver. *Amer. J. Roentgenol.*, **35**, 65-69.

Morton, C. A. (1897). *Bristol med.-chir. J.*, **15**, 323-324.

Ochsner, A., DeBakey, M., and Murray, S. (1938). Pyogenic abscess of liver; analysis of 47 cases with review of literature. *Amer. J. Surg.*, **40**, 292-319.

Pancoast, H. K. (1926). Roentgenological diagnosis of liver abscess with or without subdiaphragmatic abscess. *Amer. J. Roentgenol.*, **16**, 303-320.

Rothenberg, R. E., and Linder, W. (1934). Single pyogenic liver abscess; study of 24 cases. *Surg. Gynec. Obstet.*, **59**, 31-40.

Shaldon, C. (1958). Portal pyaemia. *Brit. J. Surg.*, **45**, 375-360.

Stokes, J. F. (1960). Cryptogenic liver abscess. *Lancet*, **1**, 355-358.

Taylor, F. (1902). A case of suppurative pylephlebitis. *Guy's Hosp. Rep.*, **56**, 109-126.

Treves, F. (1894). A case of suppurative pylephlebitis in which laparotomy was performed. *Lancet*, **1**, 663-665.

Waller (1846). Quoted by Loisson.

Yates, T. M. (1953). Distortion of pyelogram by extrarenal lesion: liver abscess distortion of pyelogram. *J. Urol.* (*Baltimore*), **69**, 309-314.

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The liver in Hodgkin's disease[1]

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SYNOPSIS This is a survey of the incidence, morphology, and significance of hepatic changes in Hodgkin's disease

Hepatomegaly and abnormal results in liver function tests are frequently encountered in patients during the course of Hodgkin's disease. It is tacitly assumed in many cases that these findings are part of the general disease process. We were impressed, however, by the frequent occurrence of hepatic changes unrelated to the presence of Hodgkin's disease, and by the difficulty in distinguishing Hodgkin's disease involving the liver from other liver disease in these patients. The present study analyses 112 necropsied cases of Hodgkin's disease with clinical or laboratory evidence of liver disease during life, or both, and reviews the literature in an attempt to elucidate this difficult problem.

REVIEW OF THE LITERATURE

A brief review of the literature cannot possibly include all the papers on this subject; therefore we apologize for any oversights or omissions.

Thomas Hodgkin (1832), in his original paper, described liver involvement incidentally, and Wilks (1865) noted involvement of the liver with Hodgkin's disease in seven of his 13 cases. In a critical analysis of Hodgkin's original cases, Fox accepted the diagnosis of Hodgkin's disease involving the liver in only two cases (Fox, 1936). In succeeding years, many cases of Hodgkin's disease with pathologically proven liver involvement were recorded by different authors (Barié, 1875; Barron, 1926; Beatty, 1954; Brauneck, 1889; Coronini, 1928; Chevallier and Bernard, 1932; Craver, 1957; Fabian, 1911; Foulon, 1932; Hartfall, 1932; Gall, 1956; Jackson and Parker, 1947; Newman and Pushkin, 1951; Klein, 1955; Murchison, 1869; Reed, 1902; Rolleston, 1912; Sternberg, 1936; Symmers, 1944; Wilks, 1865). Liver involvement in Hodgkin's disease was discussed in papers by Fabian (1911), Longcope (1903), Ziegler (1911), Chevallier and Bernard (1932), Sternberg (1936), Wallhauser (1933), Jackson and Parker (1947), and others (Ackerman and del Regato, 1954; Baker and Mann, 1939; Burnam, 1926; Goldman, 1940; Hoster, Dratman, Craver, and Rolnick, 1948; Symmers, 1944; Uddströmer, 1934; Terplan and Mittelbach, 1929), and was more specifically dealt with in papers by Coronini (1928) and later by Gruber (1930), Foulon (1932), Abbatucci and Beatty (1953), and Beatty (1954).

Students of the problem describe Hodgkin's disease involving the liver at necropsy in from 10 to 60% of the cases (Table I) (Ackerman and del Regato, 1954; Jackson and Parker, 1947; Sternberg, 1936; Symmers, 1944; Wallhauser, 1933; Ziegler, 1911). Hepatomegaly was observed clinically in from 2·3 to 60% of another series of collected cases (Table I) (Baker and Mann, 1939; Burnam, 1926; Goldman, 1940; Murray, 1908; Uddströmer, 1934). Some authors, basing their opinions on necropsy material primarily, claim that hepatic involvement is a late manifestation of Hodgkin's disease (Diamond, 1958a; Pène, du Paquier, and Pène, 1955), while others maintain that silent liver involvement can be demonstrated early in the course of the disease by liver biopsy (Pène, 1952). There is a similar difference of opinion regarding the onset of hepatomegaly, some describing it as a sign of the terminal stage of Hodgkin's disease (Ackerman and del Regato, 1954), others describing hepatomegaly as a presenting sign ('...forme hepatique et forme hepatosplenomegalic de la maladie de Hodgkin') (Pène, 1952; Pène *et al.*, 1955).

Hodgkin's disease limited to the liver has been reported (Goia, 1935; Symmers, 1944), but most

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TABLE I

INCIDENCE OF HEPATIC ENLARGEMENT AND INVOLVEMENT WITH HODGKIN'S DISEASE IN PATIENTS WITH HODGKIN'S DISEASE AS REPORTED IN THE LITERATURE

Author	*No. of Cases*	*Hepatic Enlargement (%)*	*Liver Involvement at Necropsy (%)*
Ziegler (1911)	220	60·0	
Burnam (1926)[1]	173	2·3	
Longcope and McAlpin (1927)	77	50·0	
Terplan and Mittelbach (1929)	29[2]		10·0
Wallhauser (1933)[1]	1447 (collected from the literature)		50·0
Uddströmer (1934)	548	25·9	
Sternberg (1936)	52[2]		38·0
Baker and Mann (1939)	65	30·0	
Longscope (Baker and Mann, 1939)	117	47·0	
Goldman (1940)	212	30·6	
Symmers (1944)	30[2]		58·0
Jackson and Parker (1947)	63[2] (Hodgkin's granuloma)		55·0
	19[2] (Hodgkin's sarcoma)		60·0
Abbatucci and Beatty (1953)	82[2]		53·0
Ackerman and del Regato (1954)[1]			50·0
Levitan *et al.* (1961)	112[2]	79·6	66·0
	875[3]	32·5	

[1]Review article
[2]Necropsy series
[3]Among 875 patients with Hodgkin's disease reviewed

workers believe that it is almost invariably a part of a generalized process (Craver, 1957; Hoster *et al.*, 1949). Involvement of the liver is generally, but not always, cited as occurring in conjunction with involvement of the spleen (Craver, 1957; Wallhauser, 1933; Ziegler, 1911).

Coronini (1928), Gruber (1930), and Foulon (1932) distinguish the following types of hepatic involvement by Hodgkin's disease; (1) capsular, (2) nodular or granulomatous lesions localized to the portal triad, (3) diffuse, and (4) encroachment upon or invasion of blood vessels. Simonds (1926) first noted Hodgkin's lesions invading the portal vein. According to some investigators, Hodgkin's disease primarily invades the liver within the portal spaces (Schmorl, 1922; Sternberg, 1936), while others claim that it extends along the periportal spaces (Fabian, 1911; Ziegler, 1911) or along the major biliary ducts and portal vein (Chevallier and Bernard, 1932; Sternberg, 1936).

In Hodgkin's granuloma one encounters focal circumscribed lesions or yellow streaks along the course of the portal areas (Jackson and Parker, 1947), and when extensive fibrosis is present the lesions may resemble gummas (Jackson and Parker, 1947). The hepatic parenchyma surrounding the large lesions may become atrophic (Sherlock, 1955; Symmers, 1944) or may be completely destroyed.

In Hodgkin's sarcoma grayish nodules are seen scattered throughout the liver. These lesions are histologically similar to those in the lymph nodes (Jackson and Parker, 1947). The occurrence of pathological changes unrelated to Hodgkin's disease has been appreciated by others (Abbatucci and Beatty, 1953; Beatty, 1954; Brunner, 1925; Chevallier and Bernard, 1932; Ewing, 1940; Foulon, 1932; Hoster, Zanes, and von Haam, 1949; Loeper and Lebret, 1940; Longcope, 1903; Muller and Boles, 1927; Reed, 1902; Wallhauser, 1933). Specifically noted have been tuberculosis, amyloidosis, focal necrosis, fatty degeneration, fibrosis, cirrhosis, viral hepatitis, congestion, and adhesions. Simultaneous invovlement of the liver with Hodgkin's disease and tuberculosis (Loeper and Lebret, 1940; Wallhauser, 1933), fatty degeneration (Longcope, 1903; Muller and Boles, 1927; Reed, 1902) and fibrosis (Longcope, 1903; Reed, 1902; Tschekulajev, 1951) has also been reported.

Routine laboratory liver function tests have usually been of little help in the diagnosis of lymphomatous involvement of the liver (Sherlock, 1955). Bromosulphalein retention and alkaline phosphatase levels may be abnormal (Sherlock, 1955). According to some, however, the serum alkaline phosphatase level is not as helpful in Hodgkin's disease as in detecting involvement of the liver by cancer (Abbatucci and Beatty, 1953; Ariel and Shahon, 1950; Bodansky and Bodansky, 1952; Mendelsohn and Bodansky, 1952; Ross, Iber, and Harvey, 1956). A similar negative opinion has been expressed with regard to the serum glutamic oxalacetictransaminase (SGOT) level which is, however, invaluable in the diagnosis of hepatitis (Wróblewski and LaDue, 1955). Of all the laboratory tests currently in general use, liver biopsy is probably most helpful in establishing the presence of Hodgkin's disease of the liver. But it must be recognized that a negative biopsy does not necessarily exclude the presence of hepatic Hodgkin's disease (Gall, 1956; Lichtman, 1953; Pène, 1952; Pène *et al.*, 1955; Robert, Bréchot, and Ruynat, 1957; Sherlock, 1955).

MATERIAL AND METHODS

The case records of 875 patients who were seen at the Memorial Center for Cancer and Allied Disease between 1947 and 1957 and in whom a tissue diagnosis of Hodgkin's disease was made or confirmed by members of the Department of Pathology were reviewed. Three hundred patients (34·3%) of the above group presented with clinical evidence pointing to liver disease such as hepatomegaly and/or abnormal liver function tests.[1] Of this group with

[1]The cases without hepatomegaly were either jaundiced or had an abnormal bromosulphalein test.

clinical signs of liver disease and histological proof of Hodgkin's disease, 112 cases (44%) were necropsied. The present report is concerned with an analysis of the clinical and pathological data of the latter group only. The significance of the data was evaluated by the application of a corrected χ^2 test (Yates) (Hill, 1955) and the degree of significance expressed by the P value.

RESULTS

Of the 112 necropsied cases, 74 (66%) revealed Hodgkin's disease involving the liver and 38 (34%) did not. In the latter group, however, all the livers exhibited some abnormality of major or minor degree.

The weight of the liver varied between 900 and 4,900 g., the medium weight being 2,600 g. There was no significant difference in weight between those livers involved with Hodgkin's disease and those free of Hodgkin's disease.

Of the 74 cases of Hodgkin's disease directly involving the liver, 64 (86·5%) presented with diffuse infiltration, eight (10·8%) presented with nodular lesions, and two (2·7%) exhibited mixed lesions. Both lobes of the liver were involved in most cases. Hodgkin's paragranuloma was not encountered in the liver in any instance. Fifty-six cases were classed histologically as Hodgkin's granuloma of the liver, and of these 46 (82·3%) showed almost exclusive involvement of the portal triads. In two cases the lesions were limited to the liver capsule. All cases with nodular lesions were classified histologically as granuloma, and in no patient was there invasion or involvement of hepatic blood vessels by Hodgkin's granuloma.

Hodgkin's sarcoma was present in 18 of the 74 cases (24·3%) with Hodgkin's disease directly involving the liver. Of this number, 14 presented diffuse, extensive liver infiltration while the remaining four involved primarily the portal triads. In five instances, the hepatic parenchmya adjacent to the specific lesions showed marked necrosis. The sarcomatous lesions were necrotic in three instances and invasion of blood vessels was found in five. Invasion of the main trunk of the portal vein with thrombosis was encountered in one case. In another patient, marked fibrosis of a sarcomatous lesion adjacent to the portal vein resulted in portal vein thrombosis without invasion. The hepatic vein was occluded by a thrombus (Chiari's syndrome) without invasion in a case with marked constriction of the portal vein secondary to fibrosis; the portal vein was free of thrombus and the liver was small (900 g.).

Fifty-nine (79·6%) of the 74 cases of Hodgkin's disease of the liver also presented additional pathological changes (Table II). Biliary tree pathology related to the Hodgkin's disease was found in 24 patients (34%) secondary to granulomatous as well as sarcomatous lesions (Table III). Gall bladder

TABLE II

ABNORMALITIES IN LIVER UNRELATED TO HODGKIN'S DISEASE

	Group A — *No. without Hodgkin's Disease in Liver: 38 Cases*	*Group B* — *No. with Hodgkin's Disease in Liver[2]: 74 Cases*	*Total No. with Liver Abnormalities in 112 Cases*
Passive congestion[1]	21 (55·2%)	25 (33·3%)	46 (41%)
Fatty metamorphosis	9 (23·6%)	14 (18·9%)	23 (20·5%)
Haemosiderosis	6 (15·8%)	9 (12·2%)	15 (13·4%)
Portal fibrosis	—	4 (5·4%)	4 (3·6%)
Bacterial abscesses	1 (2·6%)	2 (2·7%)	3 (2·7%)
Clostridium welchii—1 case			
Multiple pyogenic—1 case			
Nocardia abscess	—	1 (1·3%)	1 (0·9%)
Moniliasis of liver capsule	—	1 (1·3%)	1 (0·9%)
Viral hepatitis	1 (2·6%)	1 (1·3%)	2 (1·8%)
Toxic hepatitis	1 (2·6%)	—	1 (0·9%)
Portal cirrhosis	5 (13·1%)	—	5 (4·8%)
Post-necrotic cirrhosis	1 (2·6%)	—	1 (0·9%)
Amyloid	1 (2·6%)	—	1 (0·9%)
Tuberculosis	2 (5·3%)	—	2 (1·8%)
Miscellaneous	13 (34·2%)	6 (8·1%)	19 (16·9%)

[1]The incidence in this finding differs between both groups A and B significantly (P=0·05).
[2]Some patients showed more than one abnormality.

TABLE III

PATHOLOGY DUE TO HODGKIN'S DISEASE IN THE BILIARY SYSTEM

	Group A — *No. without Hodgkin's Disease in Liver: 38 Cases*	*Group B* — *No. with Hodgkin's Disease in Liver: 74 Cases*	*Total No. with Disease in Biliary System in 112 Cases*
Common duct infiltration by tumour	1	4	5
Pressure on common duct by tumour	—	10	10
Obstruction of one hepatic duct by tumour due to pressure	2	2	4
Infiltration of intra-hepatic duct by tumour	—	1	1
Infiltration and pressure on common duct and one hepatic duct by tumour	—	1	1
Partial constriction of common duct and cystic duct by surrounding tumour	—	1	1
Tumour infiltrating gall bladder wall	1	4	5
Tumour infiltrating gall bladder and cystic duct	—	1	1
Total	4 (10·5%)	24 (32·4%)	28 (25·0%)

The difference in incidence in biliary tree pathology due to Hodgkin's disease between the two groups is statistically significant (P=0·05).

TABLE IV

GALL BLADDER PATHOLOGY UNRELATED TO HODGKIN'S DISEASE

	Group A	*Group B*	*Total*
	No. without Hodgkin's Disease in Liver	*No. with Hodgkin's Disease in Liver*	
Cholelithiasis	1[a]	3[1]	4
Chronic cholecystitis	1	1	2
Choledocholithiasis	1	—	1
Total	3	4	7

[1]One patient had chronic cholecystitis and cholelithiasis and at the same time Hodgkin's disease of the gall bladder.
[a]This patient also had a mucocele of the gall bladder.

pathology unrelated to Hodgkin's disease was found in only four of the 74 cases (5·4%) (Table IV).

Nineteen of the 38 cases in which Hodgkin's disease spared the liver (50%) exhibited at least one pathological lesion in the liver while the remaining 19 had multiple abnormalities (Table II). Hodgkin's disease limited to the biliary tree, without liver involvement, was seen in only four instances (10·5%); this figure was significantly lower than in the group in which the liver itself was also involved.

Associated pathological changes encountered in other organs within the bed of the portal venous system were: (1) Two instances of oesophageal varices in patients with Hodgkin's disease involving the liver parenchyma (detailed report elsewhere, Levitan, Diamond, and Craver, 1959); (2) 30 instances of congestion, infarction, or haemosiderosis of the spleen and one case of amyloidosis of the spleen and pancreas with generalized amyloidosis as well; (3) 15 cases of fibrosis, focal necrosis, or abscesses of the pancreas; (4) four benign gastric ulcers and five duodenal ulcers. The distribution of Hodgkin's lesions in various organs in general is listed in Table V.

TABLE V

ORGANS AFFECTED BY HODGKIN'S DISEASE AT TIME OF NECROPSY IN 112 CASES[1]

Organ	*No. of Cases*	*Organ*	*No. of Cases*
Spleen	76 (67·8%)	Breast	4 (3·6%)
Liver	74 (66%)	Bladder	4 (3·6%)
Lungs	39 (34·8%)	Uterus	4 (3·6%)
Bone marrow	33 (29·5%)	Pericardium	3 (2·7%)
Gastrointestinal tract	26 (23·2%)	Ovaries	3 (2·7%)
Pancreas	26 (23·2%)	Fallopian tubes	2 (1·8%)
Kidney	12 (10·7%)	Ureter	1 (0·9%)
Adrenal	12 (10·7%)	Vagina	1 (0·9%)
Diaphragm	9 (8·0%)	Brain (proper)	1 (0·9%)
Thyroid	6 (5·4%)	Dura	1 (0·9%)
Heart	4 (3·6%)	Pituitary	1 (0·9%)

[1]Most patients had several organs involved.

TABLE VI

MECHANISMS CONTRIBUTORY TO DEATH AS DETERMINED AT NECROPSY

Infection		49 cases (43·7%)
Septicaemia	4	
Organisms		
Clostridium welchii	1	
Nocardia	1	
Actinomyces	1	
Salmonella	1	
Meningitis	4	
Organisms		
Cryptococcus neoformans	2	
Pneumococcus Type I	1	
Listeria monocytogenes	1	
Pulmonary pyogenic abscess	3	
Bronchopneumonia	23	
Lobar pneumonia	1	
Tuberculosis	10	
Miliary	2	
Tuberculoma of brain	1	
Tuberculoma of lung	7	
Moniliasis of lung	2	
Pulmonary histoplasmosis	1	
Acute bacterial endocarditis (organism?)	1	
Generalized Hodgkin's disease (with debility, toxaemia)		27 cases (24·0%)
Uraemia		14 cases (12·5%)
Compression of both pelves by tumour	1	
Tumour in both kidneys (extensive)	3	
Tumour in kidney and papillary necrosis	1	
Membranous glomerulonephritis	3	
Radiation arteriolitis and acute membranous glomerulonephritis	1	
Multiple renal cysts and nephrosclerosis	1	
Chronic pyelonephritis and renal infarct	1	
Chronic pyelonephritis, membranous Glomerulonephritis and multiple calculi	1	
Cholemic nephrosis	2	
Haemorrhage		12 cases (10·7%)
Cerebral and/or subarachnoid	4	
Gastrointestinal	7	
Retroperitoneal	1	
Hepatic coma		3 cases (2·8%)
Hepatitis	2	
Hodgkin's disease of the liver	1	
Perforation of stomach due to Hodgkin's disease		2 cases (1·8%)
Congestive heart failure		2 cases (1·8%)
Constrictive pericarditis	1	
Verrucous endocarditis	1	
Brain tumour (ependymoma of fourth ventricle)		1 case (0·9%)
Hodgkin's disease of the brain		1 case (0·9%)
Amyloid		1 case (0·9%)

Factors contributing to death in this series are listed in Table VI. It is of interest to note that five patients (4·5%) died of causes apparently unrelated to Hodgkin's disease, *viz.*, hepatic coma secondary to hepatitis (two cases), congestive heart failure (two cases), and ependymoma of the fourth ventricle (one case). An important role was attributed to infection in 49 cases (43·7%), but was considered the primary cause of death in only 16 (14·3%), the presenting infection being in the form of septicaemia, meningitis, miliary tuberculosis, cerebral tuberculoma, acute endocarditis, lobar pneumonia, or pulmonary abscess. In the remaining patients, infection was considered to be a terminal event. Four patients succumbed to cerebral or subarachnoid haemorrhage, or both, in each case apparently

secondary to severe thrombocytopenia. In contrast, of seven patients in whom gastrointestinal haemorrhage was a major factor contributing to death, only one showed thrombocytopenia, the source of bleeding in the remaining six being attributable to oesophageal varices (one case), Hodgkin's disease of the stomach (two cases), or duodenal ulcer (three cases). Uraemia was a frequent terminal event (14 cases), and membranous glomerulonephritis was encountered in five of these cases. Gastric perforation secondary to Hodgkin's disease was seen in two cases. Generalized Hodgkin's disease, with debility and 'toxaemia' in the absence of any specific additional cause, was considered the cause of death in 26 cases (24%). Only one patient in the present series was considered to have died as a direct consequence of Hodgkin's disease of the liver.

Seventeen cases of other neoplastic growths (15·2%) were encountered in the total necropsy series (Table VII).

TABLE VII

TUMOURS UNRELATED TO HODGKIN'S DISEASE IN 112 PATIENTS

Carcinomas		4
Adenocarcinoma of rectum	1	
Adenocarcinoma of colon	1	
Papillary carcinoma of thyroid	1	
Epidermoid carcinoma of skin	1	
Osteogenic sarcoma		1
Kaposi's sarcoma		1
Brain		4
Meningioma of pons	1	
Ependymoma of fourth ventricle	1	
Pinealoma	1	
Myoblastoma of pituitary	1	
Mycosis fungoides		1
Adrenal adenoma		2
Haemangiomata of liver		3
Benign ovarian cyst		1
Total		17

The clinical data in the necropsied group are arrayed, for comparison, into Group A comprising cases with Hodgkin's disease not involving the liver, and Group B cases with Hodgkin's disease involving the liver, and the combined total series.

Sex and age distribution is given in Table VIII. The ratio of males to females in the total series is 1·6 : 1. With one exception all the patients were white. The sex distribution in this series is identical with that reported in whites with Hodgkin's disease dying in 1956 for the entire United States (U.S. Dept. of Health, Education and Welfare, 1958). The youngest patient in our series was aged 15, the oldest aged 78. There was no significant difference in sex distribution between patients with Hodgkin's disease involving the liver and those without such involvement. The clinical diagnosis of histological subtype of Hodgkin's disease, based on tissue biopsy during life, is listed in Table IX. There was no significant difference in distribution of histological types of Hodgkin's disease between Groups A and B. The first clinical evidence of liver disease in relation to the time of diagnosis of Hodgkin's disease was well documented in 74 cases (66·1%) of the total series (Table Xa). Over half (56·2%) of these developed clinical evidence of liver disease within 24 months of diagnosis of Hodgkin's disease, while 73·8% developed such evidence within 36 months.

Although liver pathology was often suspected, a clinical diagnosis of a specific type of liver disease was difficult, and a diagnosis of specific liver disease was not overtly expressed in 88 cases (78·5%) of the necropsy group. In the remaining cases, Hodgkin's disease involving the liver was diagnosed in 17 (18·2%), viral hepatitis in five (4·5%), toxic hepatitis in one (0·9%), and passive congestion of

TABLE VIII

AGE[1] AND SEX[2] DISTRIBUTION

Age	*Group A*				*Group B*				*Ratio*	*No. in Total Series of 112 Cases*			
	No. without Hodgkin's Disease in Liver				*No. with Hodgkin's Disease in Liver*				*Group A:B*				
	Males	*Females*	*M/F Ratio*	*Total*	*Males*	*Females*	*M/F Ratio*	*Total*		*Males*	*Females*	*M/F Ratio*	*Total*
15-19	6	0	∞	6	1	2	0·5	3	2·0	7	2	3·5	9
20-29	4	7	0·57	11	6	4	1·5	10	1·1	10	11	0·91	21
30-39	7	7	1·00	14	17	7	2·4	24	0·58	24	14	1·7	38
40-49	1	3	0·33	4	8	5	1·6	13	0·34	9	8	1·1	17
50-59	1	1	1·0	2	10	4	2·5	14	0·14	11	5	2·2	16
60-69	0	1	0·0	1	5	2	2·5	7	0·14	5	3	1·7	8
70-79	0	0	0·0	0	3	0	3·0	3	0·0	3	0	3·0	3
Total	19	19	1·0	38	50	24	2·08	74	0·51	69	43	1·6	12

[1]The youngest patient in our series was 15 years old.
[2]The sex distribution between Group A and Group B is not statistically significant as determined by the χ^2 (corrected) test.

TABLE IX

HISTOLOGICAL DIAGNOSIS AS DETERMINED AT BIOPSY[1]

	No. in Total Necropsy Series of 112 Cases	*Group A*	*Group B*
		No. without Hodgkin's Disease in Liver in 38 Cases	*No. with Hodgkin's Disease in Liver in 74 Cases*
Hodgkin's granuloma	104 (93·0%)	36 (34·5[2], 94·8%[3])	68 (65·5[2], 91·9%[4])
Hodgkin's sarcoma	5 (4·4%)	1 (20[5], 2·6%[3])	4 (80·0[5], 5·4%[4])
Hodgkin's paragranuloma	3 (2·6%)	1 (33·3[6], 2·6%[3])	2 (66·7[6], 2·7%[4])

[1]The biopsies were performed before the onset of liver abnormalities.
[2]Percentage of 104 cases of Hodgkin's granuloma
[3]Percentage of 38 cases
[4]Percentage of 74 cases
[5]Percentage of 5 cases of Hodgkin's sarcoma
[6]Percentage of 3 cases of Hodgkin's paragranuloma

TABLE Xa

DURATION FROM DIAGNOSIS OF HODGKIN'S DISEASE TO CLINICAL EVIDENCE OF LIVER ABNORMALITY IN 74 CASES

Time in Months	*No. of Cases*
1-6	17 (23·0%)
7-12	8 (10·8%)
13-18	8 (10·8%)
19-24	9 (12·2%)
25-30	6 (8·1%)
31-36	6 (8·1%)
37-42	3 (4·1%)
43-48	4 (5·4%)
49 or more	13 (17·5%)

the liver in one (0·9%). Two of the 17 cases diagnosed during life as having Hodgkin's disease of the liver were not so confirmed at necropsy. (Choledocholithiasis, fatty liver, and haemangioma were present in one, and hepatomegaly of undetermined aetiology in the other.) Only two of the five cases diagnosed in life as viral hepatitis gave evidence of viral hepatitis at necropsy, and one of these exhibited Hodgkin's disease in the liver as well. It is possible that the remaining three cases diagnosed as viral hepatitis had healed, without pathological residua, by the time of death.

Liver disease was suspected clinically, before any form of therapy for Hodgkin's disease, in 25 cases (22·3%) All other patients in whom liver disease was suspected had received radiotherapy, chemotherapy, or steroids, alone or in combination, before the advent of signs suggestive of liver disease. Seventy-five patients (67·8%) received blood transfusions before the onset of liver disease. Six patients (5·4%) were known alcoholics, and five of these had portal cirrhosis at necropsy. Clinical symptoms and signs, and laboratory findings in both Groups A and B are listed in Tables X, XI, and XII. The degree of

TABLE X

SYMPTOMS AND SIGNS AT TIME OF ONSET OF LIVER DISEASE

	Total Necropsy Group of 112 Cases[1]	*Group A*	*Group B*
		No. without Hodgkin's Disease in Liver in 38 Cases[2]	*No. with Hodgkin's Disease in Liver in 74 Cases*[3]
Fever	94 (84·0%)	32 (84·1%)	62 (84·0%)
Anaemia	89 (79·5%)	24 (60·5%)	65 (88·0%)
Hepatomegaly	89 (79·5%)	29 (76·4%)	70 (94·6%)
Splenomegaly	63 (56·2%)	21 (55·2%)	42 (56·8%)
Jaundice	57 (50·9%)	15 (39·5%)	42 (56·8%)
Pruritus	45 (40·0%)	11 (28·9%)	34 (46·0%)
Bleeding manifestations	42 (37·6%)	19 (50·0%)	23 (32·1%)
Peripheral oedema	35 (31·3%)	8 (21·1%)	27 (36·5%)
Ascites	34 (30·4%)	11 (28·9%)	23 (31·1%)
Abdominal pain	16 (14·3%)	7 (18·4%)	9 (12·2%)
Light stools	12 (10·7%)	2 (5·3%)	10 (13·5%)
RUQ pressure	11 (9·8%)	4 (10·5%)	7 (9·4%)
Indigestion	11 (9·8%)	5 (13·2%)	6 (8·1%)
Dark urine	11 (9·8%)	2 (5·3%)	9 (12·2%)
Melaena	10 (8·9%)	3 (7·2%)	9 (9·4%)
Abnormal sugar tolerance	8 (7·2%)	2 (5·3%)	6 (8·1%)
Haematemesis	6 (5·3%)	2 (5·3%)	4 (5·4%)
Foetor hepaticus	2 (1·8%)	1 (2·6%)	1 (1·3%)
Spider naevi	1 (0·9%)	1 (2·6%)	—

[1]Percentage represents the incidence of signs or symptoms in the whole necropsy group. Many patients had multiple symptoms.
[2]Percentage represents the incidence of signs or symptoms in the 38 cases which had liver pathology other than Hodgkin's disease. Two patients in this group had extrahepatic obstructive jaundice.
[3]Percentage in this column represents incidence of signs or symptoms in the 74 cases with Hodgkin's disease involving the liver.
The difference of incidence of symptoms between Groups A and B were determined by the χ^2 test. The differences, however, were not statistically significant.

TABLE XI

PERIPHERAL BLOOD

	No. in Total Necropsy Group of 112 Cases	*Group A*	*Group B*
		No. without Hodgkin's Disease in Liver in 38 Cases	*No. with Hodgkin's Disease in Liver in 74 Cases*
Anaemia[1]	88 (79·5%)	24 (63·1%)	65 (88·0%)
Leucocytosis[2]	39 (34·8%)	14 (36·8%)	25 (33·8%)
Leucopenia[3]	15 (13·4%)	2 (5·3%)	13 (17·6%)
Thrombocytosis[4]	11 (9·8%)	3 (7·9%)	8 (10·8%)
Thrombocytopenia[5]	26 (23·2%)	10 (26·3%)	16 (21·6%)

[1]Anaemia, less than 11 g. % haemoglobin
[2]Leucocytosis, over 10,000 white blood cells per mm.[3]
[3]Leucopenia, less than 4,000 white blood cells per mm.[3]
[4]Thrombocytosis, more than 400,000 platelets per mm.[3]
[5]Thrombocytopenia, less than 90,000 platelets per mm.[3]

clinical hepatomegaly or splenomegaly, or both, and the sex distribution of cases with clinical splenomegaly are listed in Tables XIII, XIV, and XV.

There was no statistically significant difference between Groups A and B with respect to incidence of symptoms and signs, abnormal results in liver function tests, and the presence or degree of clinical

TABLE XII

LIVER FUNCTION TESTS

Test	*Group A*		*Group B*		*Total No. Cases Tested*	*Percentage Abnormal*
	No. of Cases Tested	*Percentage Abnormal*	*No. of Cases Tested*	*Percentage Abnormal*		
Bromsulphalein retention	10	100·0	23	100·0	33	100·0
Prothrombin time	8	100·0	23	91·4	31	93·5
Alkaline phosphatase	26	65·4	53	85·0	79	78·5
Total protein	31	83·9	49	85·6	80	85·0
Serum albumin	29	91·0	48	79·0	70	82·9
Serum globulin	22	27·3	48	18·7	70	22·4
Total serum bilirubin	21	52·4	62	56·4	83	55·4
Cephalin flocculation	27	63·0	61	54·1	88	56·8
Thymol turbidity	24	41·7	50	26·0	74	31·1
Total cholesterol	13	30·8	24	25·0[1]	37	27·0
Serum glutamic oxaloacetic transaminase (SGOT)	4	100·0	20	40·0	24	50·0

Abnormal Values
Bromsulphalein test, retention of 8% or more of injected dye after 45 minutes
Prothrombin time, 16 seconds or more
Alkaline phosphatase, 5 Bodansky units or more
Total protein, below 6·0 g./100 ml.
Serum albumin, below 4·0 g./100 ml.
Serum globulin, below 1·0 g./100 ml. or above 3·0 g./100 ml.
Total serum bilirubin, more than 1·5 mg./100 ml.
Cephalin flocculation, 3+ or more after 48 hours
Thymol turbidity, more than 5·0 units

[1]In two cases the values were below 100 mg./100 ml. and in two above 300 mg./100 ml. In the latter two cases, obstructive jaundice was present.
The differences in incidence of abnormal tests between Groups A and B were not statistically significant.

TABLE XIII

DEGREE OF CLINICAL HEPATOMEGALY

Cm. below Right Costal Margin	*No. in Total Necropsy Group of 112 Cases*	*Group A*	*Group B*
		No. without Hodgkin's Disease in Liver in 38 Cases	*No. with Hodgkin's Disease in Liver in 74 Cases*
1-1·99	14 (12·5%)	7 (18·4%)	7 (9·4%)
2-2·99	13 (11·6%)	5 (13·1%)	8 (10·8%)
3-3·99	12 (10·7%)	4 (10·5%)	8 (10·8%)
4-4·99	17 (15·2%)	1 (2·6%)	16 (21·6%)
5-5·99	4 (3·6%)	1 (2·6%)	3 (4·1%)
6-6·99	5 (4·5%)	1 (2·6%)	4 (5·4%)
7 or more but above pelvic rim	12 (10·7%)	8 (21·1%)	4 (5·4%)
Below pelvic rim	10 (8·9%)	None	10 (13·5%)
No clinical hepatomegaly	23 (20·4%)	9 (23·7%)	14 (18·9%)
Degree of hepatomegaly not stated	2 (1·8%)	2 (5·2%)	None

TABLE XIV

DEGREE OF SPLENOMEGALY

Cm. below Right Costal Margin	*No. in Total Necropsy Group of 112 Cases*	*Group A*	*Group B*
		No. without Hodgkin's Disease in Liver in 38 Cases	*No. with Hodgkin's Disease in Liver in 74 Cases*
Tip palpable only	10 (8·9%)	5 (13·2%)	5 (6·8%)
1-1·99	8 (7·1%)	None	8 (10·8%)
2-2·99	7 (6·2%)	2 (5·3%)	5 (6·8%)
3-3·99	10 (8·9%)	4 (10·5%)	6 (8·1%)
4-4·99	10 (8·9%)	3 (7·9%)	7 (9·5%)
5-5·99	5 (4·5%)	None	5 (6·8%)
6-6·99	3 (2·7%)	None	3 (4·0%)
7-7·99	3 (2·7%)	None	3 (4·0%)
8 or more	4 (3·6%)	3 (7·9%)	1 (1·4%)
Below pelvic rim	3 (2·7%)	None	3 (4·0%)
No clinical splenomegaly	49 (43·8%)	21 (55·2%)	28 (37·8%)

hepatomegaly or splenomegaly. Splenomegaly was found more commonly in males than females in this series ($P=0·01$). Clinical hepatomegaly was absent in 23 cases (20·4%) and splenomegaly was absent in 49 cases (43·8%), while 15 cases (13·4%) had neither enlarged spleen nor liver and five cases (4·5%) presented with a palpable spleen but not a palpable liver. There was no good correlation between the size of the liver or spleen estimated by palpation and the size found at necropsy. In no instance were nodules felt clinically in the liver. Scout films of the abdomen were helpful in assessing

TABLE XV

RELATION OF CLINICAL SPLENOMEGALY AND SEX

Total Necropsy Series	*Males*	*Females*	*Ratio Males/Females*
Number of cases without splenomegaly	24 (49·0%)	25 (51·0%)	0·96
Number of cases with splenomegaly	45 (71·5%)	18 (28·6%)	2·5
Total	69 (61·5%)	43 (38·4%)	1·6

The difference in sex distribution between the cases with splenomegaly and the cases without splenomegaly is significant ($P=0·01$).

the size of the liver and spleen. Radiological enlargement of the liver was noted in eight cases (7·1%) in which it was not detected clinically. In like manner, radiological enlargement of the spleen was found in six cases (5·4%) without clinically palpable splenomegaly.

Liver biopsy was performed in three cases (2·8%). The biopsy findings are compared with the final anatomical diagnosis of the liver involvement at necropsy in Table XVI. No mortality or morbidity resulted from the procedure.

TABLE XVI

COMPARISON BETWEEN LIVER PATHOLOGY AS SEEN ON LIVER BIOPSY SPECIMENS AND AT NECROPSY

Findings on Liver Biopsy	*Findings at Necropsy*
Miliary necrotizing hepatic granulomatous lesions ? Tuberculosis	Hodgkin's granuloma of the liver
Pericholangiolitic inflammation	Cholangitic obstruction of hepatic duct by Hodgkin's disease
Fibrosis	Hodgkin's granuloma with marked fibrosis

The five-year survival from onset of the diagnosis of Hodgkin's disease in the total series (112 cases) was 19·6% and the 10-year survival was 4·5%. No difference was noted between Groups A and B.

DISCUSSION

This retrospective analysis of necropsy material and correlation with clinical data is the only method currently available to assemble complete and detailed information on liver involvement in Hodgkin's disease. Nevertheless, keeping in mind the limitations imposed by the statistical bias of a retrospective study, and the fact that pathological findings at death may not reflect the dynamic changes occurring earlier in the course of the disease, we may acquire an appreciation of the problems encountered in making a precise diagnosis of liver disease, and the nature of the hepatic lesions involved, in patients with Hodgkin's disease.

The several types of Hodgkin's lesions of the liver encountered in this series confirm descriptions by other observers in all important respects (Coronini, 1928; Foulon, 1932; Gruber, 1930; Jackson and Parker, 1947; Simonds, 1926). Diffuse involvement of both lobes of the liver was most common, but the left lobe appeared more extensively involved in cases with associated Hodgkin's disease of the gastrointestinal tract. Nodular lesions were uncommon and, in this series, were seen only with granuloma. Although Hodgkin's sarcoma was found in only 4·4% of the diagnostic biopsy material, it was found in the liver in 24·3% of the 74 cases of hepatic Hodgkin's disease and in 21·5% of the entire necropsy series, illustrating the fact that Hodgkin's granuloma may change to sarcoma during the course of the process. This conversion took place in 19 instances. No significant difference was found between Hodgkin's sarcoma and Hodgkin's granuloma in the frequency of liver involvement.

The involvement of the liver, when present, was much more extensive in the case of Hodgkin's sarcoma than in granuloma and was characterized by massive invasion, blood vessel invasion, and marked atrophy or necrosis in the normal tissue adjacent to the lesions. In the case of Hodgkin's granuloma, on the other hand, diffuse fibrosis of a degree so marked as to obscure the underlying process was encountered.

The frequent occurrence of other liver pathology, particularly passive congestion, fatty metamorphosis, and haemosiderosis, in all cases of Hodgkin's disease with or without direct involvement of the liver by the primary process, is a striking and important finding. Passive congestion occurred less frequently in livers directly involved with Hodgkin's disease ($P=0·05$), suggesting that it was unrelated to intrahepatic invasion by Hodgkin's disease. No significant difference was found between the several other categories of hepatic abnormality in relation to the presence or absence of direct involvement by Hodgkin's disease. There is no evidence, therefore, to permit any conclusions regarding local factors predisposing the liver to involvement with Hodgkin's disease.

Passive congestion of the liver, which appeared to be a terminal event in a few instances secondary to congestive heart failure or portal hypertension, was not explicable in most instances by such mechanisms.

The presence of fatty metamorphosis of the liver in an unusually high incidence (20·5%) suggests the operation of nutritional, metabolic or toxic factors, or all three, as well as anoxia (Popper and Schaffner, 1957). All four of these mechanisms are readily demonstrated or deduced to be present in patients with advanced Hodgkin's disease. The presence of pathological changes in the pancreas in 36·6% of the cases (Hodgkin's disease 23·2%, other diseases 13·4%) may have contributed to the development of fatty liver as well (Woldman, Fishman, and Segal, 1959).

All patients with haemosiderosis of the liver (13·4%) presented with anaemia, with or without haemolysis, and had received numerous blood transfusions during life. They probably represented, therefore, secondary haemosiderosis.

In the present series, hepatic involvement with Hodgkin's disease appeared to be part of the generalized process, as has been previously maintained (Craver, 1957; Hoster *et al.*, 1948), and in no instance was Hodgkin's disease limited to the liver alone, as reported in a few cases (Goia, 1935; Symmers, 1944). Extrahepatic biliary pathology due to Hodgkin's disease was more frequent in cases with associated liver involvement ($P=0.05$), suggesting that the portal areas provide a preferred channel for direct dissemination of the process.

The spleen was involved by Hodgkin's disease in 78·4% of the cases with hepatic Hodgkin's disease. Other pathological changes were encountered in the spleen in 18·9%, and normal spleens were observed in only 2·7% of patients with liver involvement. This series confirms the conclusion that '...seldom can the liver be involved and the spleen not' (Ziegler, 1911).

Occlusion of the portal or hepatic vein by associated thrombosis, direct tumour compression or blood vessel invasion (7·2% of this series) must be considered in the differential diagnosis of the onset of ascites, jaundice, or liver enlargement in patients with hepatic involvement with Hodgkin's disease.

The incidence of liver involvement by Hodgkin's disease is somewhat higher (80%) in the necropsy series than reported by others (Table I), an expected finding in view of the method of selection of the material.

The sex distribution of patients with Hodgkin's disease of the liver did not differ significantly from the cases without liver involvement or from the expected sex distribution in Hodgkin's disease at large (Diamond, 1958c; Gall and Mallory, 1942; U.S. Department of Health, Education and Welfare, 1958; Wallhauser, 1933). This is in contrast to the report of Abbatucci and Beatty (1953) that males more frequently exhibited hepatic involvement than could be anticipated on the sex distribution of Hodgkin's disease in general. A critical analysis of the data on which their conclusion was based (31 males and 13 females with liver involvement and 29 males and 19 females without liver involvement in a total series of 82 cases) by the corrected χ^2 test (Yates) (Hill, 1955) reveals no significant difference from the sex distribution of a number of series of cases of generalized Hodgkin's disease (Diamond, 1958b; Gall, 1956; U.S. Dept. of Health, Education and Welfare, 1958; Wallhauser, 1933). There is no basis, therefore, for assuming a hormonal influence on the frequency of hepatic involvement with Hodgkin's disease.

Massive hepatomegaly, with the liver edge below the pelvic brim, was seen in 8·9% of the necropsy series, an exception to the statement that marked hepatomegaly is uncommon or rare in Hodgkin's disease (Chevallier and Bernard, 1932; Ziegler, 1911). In all cases with massive hepatic enlargement, Hodgkin's disease was found in the liver at necropsy. Sixty-eight per cent of our series exhibited lesser degrees of hepatomegaly, however, and within this group only 52% had Hodgkin's disease involving the liver directly. However, in those cases without clinical evidence of hepatomegaly (20·4%) of the necropsy series), Hodgkin's disease was found in 60·8%. It would appear, therefore, that with the exception of massive hepatomegaly, there is no good correlation between hepatic enlargement and the presence of Hodgkin's disease in the liver. This may be due, in part, to the lack of correlation between clinical hepatomegaly in life and hepatic weight at necropsy, an observation made with respect to other disease as well and considered to be due to differences in blood content of the organ in life and after death (Schiff, 1957).

Although it is stated that massive hepatomegaly in Hodgkin's disease is almost always associated with massive splenomegaly (Chevallier and Bernard, 1932; Craver, 1957), no good correlation was noted in the present series between the degree of hepatomegaly and the presence or degree of associated splenomegaly. Indeed, splenomegaly was absent in 30·4% of the cases with hepatomegaly in this series. As with the liver, there was no good correlation between the clinical estimate of splenomegaly and the size of the spleen at necropsy, or between the size of the spleen and the presence of Hodgkin's disease within the spleen.

Splenomegaly was encountered more frequently in males than females both in the necropsy series ($P=0.01$) and in a larger series of 300 cases of Hodgkin's disease with clinical evidence of liver disease. Such an observation has not been previously recorded but its significance is obscure.

It is clear from this series that hepatomegaly can occur early in the course of Hodgkin's disease and is not limited to the terminal stages as claimed by some (Ackerman and del Regato, 1954). A comparison of the duration of life from the time of diagnosis of Hodgkin's disease with, and without, hepatomegaly reveals no significant difference. The five- and 10-year survival in our series did not differ statistically from a series of 713 cases reported by Diamond (1958b) from the Memorial Center, in which survival was calculated from the first hospital visit rather than from the time of diagnosis of Hodgkin's disease. Although the interval from the first hospital visit to death is not identical with the interval from diagnosis to death, the difference was small generally and could not significantly influence

the five- and 10-year survival time. Survival in Diamond's series was 19·5% at five years and 7·2% at 10 years in comparison with 19·6% and 4·5% respectively in this series.

It is noteworthy that, despite the fact that all the patients in our series had liver abnormalities, only three patients (2·8%) died in hepatic coma, two as the result of intercurrent viral hepatitis and only one directly attributable to Hodgkin's disease of the liver.

Liver disease becomes suspect in the patient with Hodgkin's disease by the usual clinical (or radiological) criteria of liver enlargement, jaundice, ascites, peripheral oedema, or abnormal liver function tests. Among the liver function tests, the most useful, in order of frequency of abnormal findings, were bromosulphalein (BSP) retention, prothrombin time, total protein, serum albumin, alkaline phosphatase, cephalin flocculation, serum bilirubin, and thymol turbidity. However, since the incidence of specific abnormalities and clinical signs did not differ significantly in those patients with Hodgkin's disease of the liver from those with other forms of liver disease, these tests fail to distinguish the two groups, findings in agreement with those of other authors (Abbatucci and Beatty, 1953; Sherlock, 1955). Only those patients with massive hepatomegaly (below the pelvic brim) had uniform involvement with Hodgkin's disease, suggesting that this degree of hepatic enlargement is of clinical value in evaluating the state of the liver.

Liver biopsy was performed too infrequently (2·8%) in the present series to permit evaluation of its importance. Haemorrhagic tendencies, a not uncommon manifestation of advanced Hodgkin's disease, particularly after various forms of therapy, prohibited the use of liver biopsy in many instances.

The difficulty in establishing a correct and precise clinical diagnosis of liver pathology in Hodgkin's disease is clearly due to the multiplicity of type of lesions that may be encountered at the same time within the liver and the biliary tree in any one case. The correct diagnosis, however, is not simply of academic interest, particularly in those instances in which choledocholithiasis, hepatitis, cirrhosis, or bacterial infections were present. Radiotherapy or chemotherapy, in such instances, may further complicate the problem. On the other hand, it is equally poor care to withhold specific therapy in the patient who is jaundiced because of tumorous lymph nodes obstructing the extrahepatic bile ducts, where radiotherapy may offer palliation of the very disturbing pruritus (Levitan *et al.*, 1961). Furthermore, any evaluation of the efficacy of a method of therapy for Hodgkin's disease of the liver is obviously impossible without a correct diagnosis at the onset of therapy. Therefore, every effort should be made to establish, in detail, the cause of liver disease before the institution of therapy in the patient with Hodgkin's disease. A high index of suspicion of the possibility of unrelated liver disease in the patient with established diagnosis of Hodgkin's disease, combined with full utilization of liver function tests, liver biopsy (when feasible), intravenous cholangiography, and/or lienoportography (when feasible) may contribute to a more accurate antemortem diagnosis. Evaluation of new diagnostic tests, such as serum iron and serum B_{12} determinations, and enzyme assays (Grossowicz, Hochman, Aronovitch, Izak, and Rachmilewitz, 1957), and radio-tracer scanning of the liver (Friedell, MacIntyre, and Rejali, 1957) may uncover important clues leading to improved diagnosis. Serial liver function tests or serial liver biopsies may contribute greatly to our understanding of the relationship between Hodgkin's disease and intercurrent liver disease as a dynamic process.

SUMMARY

Three hundred (34·3%) of 875 patients with histologically proven Hodgkin's disease had clinical evidence of associated liver disease. One hundred and twelve cases of the 300 (37%) were subject to necropsy and critical clinical and pathological study. In the necropsy series, 66% had direct involvement of the liver with Hodgkin's disease, and 34% exhibited other pathology in the liver.

Seventy-nine per cent of the patients with Hodgkin's disease of the liver also had other pathological changes in this organ, and 32·4% had biliary tract disease as well.

Diffuse involvement of the liver by Hodgkin's disease was the most common type of intrahepatic distribution (86·5%), whereas discrete nodular lesions were found in only 10·8% and the combined diffuse and nodular type was encountered in only 2·7%.

Hodgkin's granuloma was most frequently localized to the portal triads and was not seen invading the blood vessels.

Hodgkin's sarcoma tended to involve the liver in a more diffuse and extensive manner and frequently invaded the blood vessels.

The larger bile ducts were invaded or compressed by Hodgkin's granuloma or sarcoma in 21·8% of the cases with liver involvement.

In patients free of Hodgkin's disease in the liver itself but with histological evidence of Hodgkin's disease elsewhere, pathological changes of some kind were found in all livers. Some combination of

chronic passive congestion, fatty metamorphosis, or haemosiderosis was encountered in 50% of such cases; biliary tree pathology due to Hodgkin's disease in 10·5%; and unrelated biliary tree pathology in 6·2%. Statistical analysis of the incidence of hepatic changes indicated no significant correlation of these findings with the presence or absence of direct involvement of the liver by Hodgkin's disease.

Despite the almost uniform finding of liver pathology in patients with Hodgkin's disease in this series, only 2·8% of the patients died as a consequence of clinical liver failure, one attributable to Hodgkin's disease directly and two to intercurrent hepatitis.

The five- and 10-year survival rate in this series, selected on the basis of clinical evidence of hepatic disease, did not differ significantly from the survival rate of Hodgkin's disease in general as encountered at Memorial Hospital. The presence or absence of hepatomegaly, the degree of hepatomegaly, splenomegaly, and sex did not significantly influence survival.

At the present time, on the basis of clinical findings and currently available liver function tests, it is impossible to distinguish reliably between liver disease due to Hodgkin's disease itself and that due to associated hepatic changes. Perhaps the only reliable sign of direct involvement of the liver in the Hodgkin's process is the enlargement of the liver below the pelvic brim.

Statistical analysis of the sex distribution of involvement with Hodgkin's disease failed to confirm a previous report of predilection of males to hepatic Hodgkin's disease. By similar statistical criteria, however, splenic involvement with Hodgkin's disease was more frequent, in our series, than the anticipated incidence. The significance of this observation is obscure.

Clinical hepatomegaly was encountered in 79·5% of the necropsied series of patients with Hodgkin's disease selected on the basis of evidence of liver disease, and it was seen relatively early, in some instances, in the course of the Hodgkin's process.

It is concluded that hepatic involvement by Hodgkin's disease is but one manifestation of a generalized disease process, that it carries no prognostic significance for survival, that it may occur early or late, and that it is frequently associated with one or more abnormalities of liver structure which may be unrelated to Hodgkin's disease. With the single exception of massive hepatomegaly, no clinical sign establishes the presence of the Hodgkin's process within the liver itself, and liver biopsy, the only currently available test capable of the definitive diagnosis of hepatic Hodgkin's disease, is often interdicted by complicating factors.

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REFERENCES

Abbatucci, J. S., and Beatty, E. C. (1953). Atteinte hépatique dans la maladie de Hodgkin. Différences dans les deux sexes. *Bull. Ass. franç. Cancer*, **40**, 371-382.

Ackerman, L. V., and del Regato, J. A. (1954). Cancer: Diagnosis, Treatment and Prognosis, 2nd ed. Mosby, St. Louis.

Ariel, I. M., and Shahon, D. B. (1950). Hepatic dysfunction in candidates for abdominal surgery, especially in patients with cancer. *Cancer*, **3**, 608-623.

Baker, C., and Mann, W. N. (1939). Hodgkin's disease. A study of 65 cases. *Guy's Hosp. Rep.*, **89**, 83-107.

Barié, E. (1875). Lymphadénie sans leucémie; hypertrophie généralisée des ganglions lymphatiques tumeurs lymphoïdes du foie, de la rate, des reins, du coeur, de la peau, etc. *Bull. Soc. anat. Paris*, **50** (3 ser., **10**), 711-719.

Barron, M. (1926). Two rare cases of Hodgkin's disease: (a) chronic relapsing fever of Pel-Ebstein type; (b) Hodgkin's disease with extensive cutaneous manifestations. *Arch. Path.* (*Chicago*), **2**, 616.

Beatty, E. C., Jr. (1954). Jaundice in Hodgkin's disease. *Bull. N.Y. Acad. Med.*, **30**, 409-412.

Bodánsky, M., and Bodansky, O. (1952). *Biochemistry of Disease*, 2nd ed. Macmillan, New York.

Brauneck, H. (1889). Über einen Fall von multipler Lymphombildung (Hodgkin'scher Krankheit), welcher unter hohem Fieber, Icterus und Nephritis zum Tode führte. *Dtsch. Arch. klin. Med.*, **44**, 297-310.

Brunner, A. (1925). Ein Fall von Lymphogranulomatose mit stürmischem Verlauf. *Wien. klin. Wschr.*, **38**, 930-932.

Burnam, C. F. (1926). Hodgkin's disease, with especial reference to its treatment by irradiation. *J. Amer. med. Ass.*, **87**, 1445-1452.

Chevallier, P., and Bernard, J. (1932). *La maladie de Hodgkin*. Masson, Paris.

Coronini, C. (1928). Über das Paltauf-Sternberg'sche Lymphogranulom, mit besonderer Berücksichtigung der Veränderungen des Verdauungsschlauches und solcher an der Leberpforte mit und ohne Gelbsucht. Zugleich ein Beitrag über lymphogrnaulomatöse Gefässveränderungen. *Beitr. path. Anat.*, **80**, 405-478.

Craver, L. F. (1957). Hodgkin's Disease. In *The Practice of Medicine* Vol. 5. Edited by L. Hendrick Sloan. Hagerstown, Md.

Diamond, H. D. (1958a). The Lymphomas. *Disease-a-Month*, pp. 38-60.

—— (1958b). Results of therapy in Hodgkin's disease. *Ann. N.Y. Acad. Sci.*, **73**, 357-362.

—— (1958c). *The Medical Management of Cancer*. Grune & Stratton, New York.

Ewing, J. (1940). *Neoplastic Diseases*, 4th ed. Saunders, Philadelphia.

Fabian, E. (1911). Die Lymphogranulomatosis (Paltauf-Sternberg). *Zbl. allg. Path. path. Anat.*, **22**, 145-186.

Foulon, P. (1932). A propos de quelques lésions extra-ganglionnaires de la lymphogranulomatose. *Ann. Anat. path.*, **9**, 725-744.

Fox, H. (1936). Remarks on microscopical preparations made from some of the original tissue described by Thomas Hodgkin, 1832. *Guy's Hosp. Rep.*, **86**, 11-16.

Friedell, H. L., MacIntyre, W. J., and Rejali, A. M. (1957). A method for the visulization of the configuration and structure of the liver. Part A. Preliminary clinical investigations. *Amer. J. Roentgenol.*, **77**, 455-470.

Gall, E. A. (1956). In *Diseases of the Liver*, ed. L. Schiff. Lippincott, Philadelphia.

——, and Mallory, T. B. (1942). Malignant lymphoma. A clinicopathologic survey of 618 cases. *Amer. J. Path.*, **18**, 381-415.

Goia, I. (1935). Contributions à la forme hépatique de la lymphogranulomatose maligne. *Sang.*, **9**, 367-374.

Goldman, L. B. (1940). Hodgkin's disease; an analysis of 212 cases. *J. Amer. med. Ass.*, **114**, 1611-1616.

Grossowicz, N., Hochman, A., Aronovitch, J., Izak, G., and Rachmilewitz, M. (1957). Malignant growth in the liver and serum-vitamin-B_{12} levels. *Lancet*, **1**, 1116-1117.

Gruber, G. B. (1930). Spezielle Infektionsfolgen der Leber. IX. Lymphogranulomatose. In *Handbuch der speziellen pathologischen Anatomie und Histologie*, ed. F. Henke and O. Lubarsch, Vol. 5, Pt. I, pp. 604-615. Springer, Berlin.

Hartfall, S. J. (1932). Hodgkin's disease of the lung. *Guy's Hosp. Rep.*, **82**, 55-74.

Hill, A. B. (1955). *Principles of Medical Statistics*, 6th ed. The Lancet, London.

Hodgkin, T. (1832). On some morbid appearances of the absorbent glands and spleen. *Med.-chir. Trans.*, **17**, 68-114. London. Reprinted in *Med. Classics*, **1**, 741-770 (1937).

Hoster, H. A., Zanes, R. P., Jr., and Haam, E. von (1949). The association of 'viral' hepatitis and Hodgkin's disease (a preliminary report). *Cancer Res.*, **9**, 473-480.

—, Dratman, M. B., Craver, L. F., and Rolnick, H. A. (1948). Hodgkin's disease (1832-1947). *Ibid*, **8**, 1-78.

Jackson, H., and Parker, F. (1947). *Hodgkin's Disease and Allied Disorders*. Oxford Univ. Press, New York.

Klein, F. (1955). Über einen Fall von akut verlaufender Lymphogranulomatose. *Zbl. allg. Path. path. Anat.*, **93**, 19-27.

Levitan, R., Diamond, H. D., and Craver, L. F. (1959). Esophageal varices in Hodgkin's disease involving the liver. *Amer. J. Med.*, **27**, 137-143.

—, —, — (1961). Jaundice in Hodgkin's disease. *Ibid*, **30**, 99-111.

Lichtman, S. S. (1953). *Diseases of the Liver, Gallbladder and Bile Ducts*, 3rd ed. Vol. 2. Lea & Febiger, Philadelphia.

Loeper, M., and Lebret, G. (1940). Association de tubercules et l'infiltration granulomateuse dans le foie d'une maladie de Hodgkin terminée par ictère. *Bull. Soc. méd. Hôp. Paris*, 3 ser., **56**, 492-498.

Longcope, W. T. (1903). On the pathological histology of Hodgkin's disease, with a report of a series of cases. *Bull. Ayer clin. Lab.*, **1**, 4-76.

—, and McAlpin, K. R. (1927). In *A Textbook of Medicine by American Authors*. Saunders, Philadelphia.

Mendelsohn, M. L., and Bodansky, O. (1952). The value of liver-function tests in the diagnosis of intrahepatic metastases in the nonicteric cancer patient. *Cancer*, **5**, 1-8.

Muller, G. P., and Boles, R. S. (1927). Abdominal manifestations of Hodgkin's disease. *J. Amer. med. Ass.*, **88**, 301-307.

Murchison, C. (1869). "Lymphatic new formations" in the liver, with enlargement of the spleen and persistent jaundice, in a child aged 12. *Trans. path. Soc. Lond.*, **20**, 198-203.

Murray, G. R. (1908). Lymphadenoma. In *A System of Medicine*, ed. C. Allbutt and H. D. Rolleston, Vol. 4, Pt. I, pp. 459-481. Macmillan, London. Cited in Fabian, E. (1911).

Newman, B., and Pushkin, W. (1951). Acute fulminating Hodgkin's disease. *J. Amer. med. Ass.*, **146**, 335-337.

Pène, P. (1952). Foie et lymphogranulomatose de Hodgkin. *Gaz. méd. Fr.*, **59**, 1245-1250.

Pène, P., Paquier, P. du, Alium, and Pène, J. A. (1955). Forme hépato-splénomégalique prédominante de la maladie de Hodgkin. *Bordeaux chir.*, **2**, 93-94.

Popper, H., and Schaffner, F. (1957). *Liver: Structure and Function*. McGraw-Hill, New York.

Reed, D. M. (1902). On the pathological changes in Hodgkin's disease, with especial reference to its relation to tuberculosis. *Johns Hopk. Hosp. Rep.*, **10**, 133-196.

Robert, Bréchot, and Ruynat (1957). A propos d'une forme hépatique de la maladie de Hodgkin. *Arch. Mal. Appar. dig.*, **46**, 822-828.

Rolleston, H. D. (1912). *Diseases of the Liver, Gallbladder and Bile-Ducts*, 2nd ed. Macmillan, London.

Ross, R. S., Iber, F. L., and Harvey, A. McG. (1956). The serum alkaline phosphatase in chronic infiltrative disease of the liver. *Amer. J. Med.*, **21**, 850-856.

Schiff, L. (1957). Absence of a palpable liver – a sign of value in excluding obstructive jaundice due to pancreatic cancer. *Gastroenterology*, **32**, 1143-1144.

Schmorl (1922). Case presentation before the Gesellschaft für Natur und Heilkunde zu Dresden, on 20 March 1922. *Münch. med. Wschr.*, **69**, 908.

Sherlock, S. (1955). *Diseases of the Liver and Biliary System*. Blackwell, Oxford.

Simonds, J. P. (1926). Hodgkin's disease. *Arch. Path. (Chicago)*, **1**, 394-430.

Sternberg, C. (1936). Lymphogranulomatose und Reticuloendotheliose. *Ergebn. allg. Path. path. Anat.*, **30**, 1-76.

Symmers, D. (1944). Clinical significance of the deeper anatomic changes in lymphoid diseases. *Arch. intern. Med.*, **74**, 163-171.

Terplan, K., and Mittelbach, M. (1929). Beiträge zur Lymphogranulomatose und zu anderen eigenartigen, verallgemeinerten Granulomen der Lymphknoten. *Virchows Arch. path. Anat.*, **271**, 759-866.

Tschekulajev, G. N. (1951). The cytodiagnosis of lymphogranulomatosis in effusions. *Klin. Med. (Mosk.)*, **29**, No. 4, pp. 60-63.

Uddströmer, M. (1934). On the occurrence of lymphogranulomatosis (Sternberg) in Sweden 1915-1931 and some considerations as to its relation to tuberculosis. *Acta tuberc. scand.*, Suppl. I, pp. 1-225.

U.S. Dept. of Health, Education and Welfare (1958). Vital Statistics—special reports. Mortality from each cause by color and sex, U.S. 1956, *Publ. Hlth Serv.*, **48**, 5.

Wallhauser, A. (1933). Hodgkin's disease—general review. *Arch. Path. (Chicago)*, **16**, 522-562 and 672-712.

Wilks, S. (1865). Cases of enlargement of the lymphatic glands and spleen (or, Hodgkin's disease), with remarks. *Guy's Hosp. Rep.*, 3 ser., **11**, 56.

Woldman, E. E., Fishman, D., and Segal, A. J. (1959). Relation of fibrosis of the pancreas to fatty liver and/or cirrhosis. An analysis of one thousand consecutive autopsies. *J. Amer. med. Ass.*, **169**, 1281-1283.

Wróblewski, F., and LaDue, J. S. (1955). Serum glutamic-oxalacetic-transaminase activity as an index of liver-cell injury from cancer. *Cancer*, **8**, 1155-1163.

Ziegler, K. (1911). *Die Hodgkinsche Krankheit*. Fischer, Jena.

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Upper gastrointestinal haemorrhage in the non-European

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SYNOPSIS An analysis of the 149 cases of haematemesis admitted to Coronation Hospital from January 1951, to September 1959, is presented. The commonest cause was found to be acute gastritis and gastric erosion. These cases were more common in males and usually followed on an alcoholic bout. These were in the main mild. Twenty-five cases associated with peptic ulceration were found, and the lesion occurred more commonly in males under the age of 40. Duodenal ulcer was commoner than gastric ulcer. The incidence of haematemesis with peptic ulceration appears to be on the increase, the cause of which is not known. Cirrhosis of the liver is a frequent cause of haematemesis and carries a high mortality.

The difficulties of diagnosis and treatment of cases of gastrointestinal haemorrhage in the European have been well documented (Avery Jones, Read, and Stubbe, 1959). The problem is much greater in the non-European patient as the haemorrhage is usually the sole presenting feature, and clinical examination is in the majority of cases negative.

The routine followed in the Medical Division of this hospital is that the history and clinical findings are evaluated as accurately as possible. A surgical consultation is arranged if the patient is clinically anaemic. Complete haematological and biochemical studies, liver function tests, and sigmoidoscopy are carried out. Radiological examinations are performed as soon as possible, preferably within seven days in order to avoid the possibility of overlooking a rapidly healing ulcer. Stools and urines are examined for parasites. Gastroscopy is not routinely performed.

In a survey of the records of this hospital from 1951 to September 1959, 149 cases of gastrointestinal haemorrhage were found to have been admitted.

TABLE I

AETIOLOGICAL GROUPS

	No. of Cases	*Deaths*
Peptic ulcer	25	2
Acute gastritis and gastric erosions	65	2
Cirrhosis and oesophageal varices	32	13
Gastric malignancy and tumours	7	1
Other causes	7	4
Total	136	22

Thirteen of these cases were Indians and these are not included in the analysis (Table I).

PEPTIC ULCERATION

All cases (Table II) were proved either by radiography, laparotomy, or necropsy. It will be seen that duodenal ulcer occurs three times as commonly as gastric ulceration, *i.e.*, 19 cases: 6 cases. Peptic ulceration occurs far more commonly in the younger age groups, 72% of the patients being under 40. This applies to both duodenal and gastric ulcers although the figures for gastric ulcer are too small for any valid deduction to be made. The male sex is predominately affected: 80% of the cases were males. It will be seen from Table III that the number of cases is increasing, especially during the last nine months.

ACUTE GASTRITIS AND GASTRIC EROSIONS

The largest number of cases (Table IV) fell under this heading. Routine investigation proved completely negative, no cause for the bleeding being found. The severity of the bleeding varied considerably, the majority of the cases being mild. Two of these cases were subjected to gastrectomy owing to continued haematemesis and the pathological findings were (1) acute gastritis with haemorrhage into the mucosa and submucosa, and (2) phlegmonous gastritis. Two patients were admitted severely bled out and died shortly after admission. Permission for

TABLE II

PEPTIC ULCERATION

Age Group (years)	*Under 30*		*30-39*		*40-49*		*50-59*		*Over 60*		*Total*
	Duodenal Ulcer	*Gastric Ulcer*	*Duodenal Ulcer*	*Gastric Ulcer*	*Duodenal Ulcer*	*Gastric Ulcer*	*Duodenal Ulcer*	*Gastric Ulcer*	*Duodenal Ulcer*	*Gastric Ulcer*	
Bantu males	1	2	6	0	3	0	1	0	0	0	13
Bantu females	2	1	–	–	–	–	–	–	–	–	3
Coloured males	1	0	1	2	1	0	1	0	1	0	7
Coloured females	1	1	–	–	–	–	–	–	–	–	2
	9		9		4		2		1		25

One case of pyloric stenosis was found.
One case of perforation was associated with haemorrhage.
Nine gastrectomies were performed.

TABLE III

NUMBER OF CASES ADMITTED ANNUALLY

Year	*1951*	*1952*	*1953*	*1954*	*1955*	*1956*	*1957*	*1958*	*1959 (9 mth.)*
No. of cases	2	0	3	0	1	2	3	4	10=25

necropsy was not obtained in either case so the final diagnosis remains obscure.

In many of these cases there was a history of alcoholic excess, the haematemesis following on persistent vomiting. Phenolphthalein, cantharides, and aspirin were each suspected of being a precipitating factor in three cases.

In this group males (68%) predominated over females, and again the majority of the patients (70%) were under the age of 40.

TABLE IV

ACUTE GASTRITIS AND GASTRIC EROSIONS

Age (years)	*Under 30*	*30-39*	*40-49*	*50-59*	*Over 60*	*Total*
Bantu males	8	9	7	3	3	30
Bantu females	6	5	1	1	–	13
Coloured males	6	6	2	0	1	15
Coloured females	5	0	1	0	1	7
Total	25	20	11	4	5	65

CIRRHOSIS AND OESOPHAGEAL VARICES

In this group males again predominated (72%); but the tendency is for the older age group to be affected (Table V). Operation was undertaken in five cases. In two cases a portocaval anastomosis was performed and in a further two, transection of the stomach was carried out.

TABLE V

CIRRHOSIS AND OESOPHAGEAL VARICES

Age (years)	*Under 30*	*30-39*	*40-49*	*50-59*	*Over 60*	*Total*
Bantu males	1	9	1	1	3	15
Bantu females	3	1	2	1	–	7
Coloured males	–	1	2	5	1	9
Coloured females	–	–	–	–	1	1
Total	4	11	5	7	5	32

GASTRIC MALIGNANCY AND TUMOURS

The following are the findings in this group:—

Gastric carcinoma	4
Leiomyosarcoma	1
Leiomyoma	1
Reticulum cell sarcoma	1
	7

OTHER CAUSES

Other causes were:—

Ruptured aortic aneurysm	2
Oesophageal carcinoma	1
Oesophageal ulceration	1
Anticoagulation therapy	1
Chronic intussusception	1
Gall stones (doubtful aetiology)	1
	7

DISCUSSION

A comparison of these figures with those in recent British publications reveals marked differences (Avery Jones *et al.*, 1959; Cates, 1959). Whereas over half their cases with haematemesis were caused by peptic ulceration, this association was found in only 18% of our cases. Peptic ulcer has long been recognized as rare in the non-European, particularly the Bantu (Eagle and Gillman, 1938), though an increasing incidence has been reported recently (Baldachin and Palmer, 1959). The increasing frequency with which haematemesis from this source is being found in this hospital tends to confirm these findings. We have noted no change in the patients

admitted with regard to race, mentality, standard of education, employment, diet, or any other factor which could possibly account for this increased frequency. The admission rate of patients has increased considerably but not in proportion to the number with peptic ulceration.

Another noteworthy feature is that bleeding in our cases of peptic ulcer occurred mainly in the under-40 age group. This is in marked contradistinction to the findings of Cates in European cases where bleeding occurred very much more commonly in the over-40 age group. Males predominated in the non-European (20 out of 25) even more than they did in the European series.

The largest group was that labelled 'acute gastritis and gastric erosion'. In contrast to the findings of Cates, which showed an equal incidence of the sexes in his acute ulcer or erosion group, most of our patients were men (45 out of 65). This is ascribed to the fact that in the majority of our cases the haematemesis followed on continuous vomiting (Mallory-Weiss syndrome) after alcoholic bouts.

We found portal hypertension to be a cause of haematemesis much more frequently than did Avery Jones *et al.* and Cates in England. Nearly one quarter of our cases fell into this group. In this respect, our figures approximate those from the United States of America. One third of the patients from the Massachusetts General Hospital with massive upper gastrointestinal haemorrhage (Welch, Allen, and Donaldson, 1955) and over 10% of all cases of haematemesis from the Boston City Hospital (Smythe, Osborne, Zamcheck, Richards, and Madison, 1957) bled from gastro-oesophageal varices. Again in Boston, gastritis is also a common cause of haemorrhage. Alcoholic consumption would appear to be a major factor in determining the similarity of our figures with American figures, and their variance with those from Britain. The prognosis in the non-European with bleeding varices is grave, as it is in the European (Sherlock, 1955). Perhaps the mortality may be lessened by a less conservative approach with more frequent and earlier surgery. However, very few of our cases would qualify for portocaval anastomosis if the criteria as laid down by Sherlock were followed, namely, adequate liver cell function, serum bilirubin less than 1·5 mg.%, serum albumin level greater than 3 g.%, and no ascites. In particular it is extremely uncommon for us to encounter a serum albumin level above 3 g.% in our cases of cirrhosis. This is not surprising, as the albumin level of the Bantu tends to the lower limit of the normal accepted for the European (Gelfand, 1958). It is felt that better results might be obtained if patients were submitted to surgery forthwith, when bleeding persists despite conservative measures including balloon tamponage. As so few of these patients are suitable candidates for portocaval anastomosis, then transection of the stomach or direct transoesophageal suture of the varices is indicated.

It appears that carcinoma of the stomach is an uncommon cause of gastrointestinal bleeding in the non-European. In only one case was haematemesis a presenting symptom in carcinoma of the oesophagus, though this is a fairly common lesion in the Bantu. No case was associated with diaphragmatic hernia. An interesting feature is that hiatus hernia has never been found in this hospital in the Bantu, and only very rarely in the Coloured.

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REFERENCES

Annotation (1959). *Brit. med. J.*, 1, 1462-1463.
Baldachin, B. J., and Palmer, P. E. S. (1959). *Ibid*, 2, 304.
Cates, J. E. (1959). *Ibid*, 1, 206-211.
Eagle, P. C., and Gillman, J. (1938). *S. Afr. J. med. Sci.*, 3, 1-6.
Gelfand, M. (1958). *Leech (Johannesburg)*, 28, 49.
Jones, F. Avery, Read, A. E., and Stubbe, J. L. (1959). *Brit. med. J.*, 1, 1138-1142.
Sherlock, S. (1955). *Diseases of the Liver and Biliary System*. Blackwell, Oxford.
Smythe, C. M., Osborne, M. P., Zamcheck, N., Richards, W. A., and Madison, W. M. (1957). *New Engl. J. Med.*, 256, 441-447.
Welch, C. E., Allen, A. W., and Donaldson, G. A. (1955). *Ibid*, 252, 921-928.

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Methods and techniques

Laboratory diagnosis of gastrointestinal bleeding

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SYNOPSIS The value of four tests to detect occult alimentary blood loss has been compared in normal subjects, in patients with and without alimentary disease, and in patients with congestive heart failure, and the sensitivities of these tests have been compared using suspensions of blood alone and blood in faeces. The Occultest proved almost as sensitive as did the benzidine test in the detection of faecal blood loss, and, if combined with a spectroscopic test for protoporphyrin, minimizes the number of 'false' positive results that are reported. Such false positive results will inevitably occur but are reduced by dietary restriction before the test is made.

We have been investigating the use of laboratory methods in the diagnosis of ulceration and neoplasm of the alimentary tract for about seven years. During this period benzidine became difficult to obtain following the observations by Case, Hosker, Drever, and Pearson (1954) on the very high incidence of neoplasm of the urinary bladder in workers engaged in the manufacture of this and certain other substances. We then decided to change to orthotolidine, which appeared to be the most popular alternative reagent for the demonstration of faecal occult blood, and about this time obtained a supply of Haematest tablets. While we were interested in studying a method less sensitive than the benzidine test, these proved too insensitive for our purpose and we were offered Occultest tablets, designed primarily for the demonstration of blood in urine. Both of these are orthotolidine preparations containing strontium peroxide.

The purpose of this paper is to describe comparative observations on a benzidine test, an orthotolidine test, and these two tablet tests, and to discuss their value and that of the spectroscopic examination of faeces for protoporphyrin in the diagnosis of alimentary bleeding.

METHODS

In the benzidine and orthotolidine tests analytical reagent quality chemicals and acid-washed glassware only are used.

BENZIDINE TEST A portion of faeces the size of a pea is suspended in 5 ml. of distilled water and boiled for five minutes. This time is not critical and boiling for up to half-an-hour does not appear to alter the sensitivity of this or the orthotolidine test. In a test-tube, to 1 in. of a saturated solution of benzidine in glacial acetic acid is added an equal volume of hydrogen peroxide, 10 vol. strength. The solutions are mixed and a little of the cooled faecal suspension is gently run on its surface. The tube is tapped to allow slight admixture and the result read after one minute.

Strong positive	deep blue ring
Moderate positive...	deep green ring
Weak positive.....	pale green ring
Negative.....	no colour change

ORTHOTOLIDINE TEST A 4% solution of orthotolidine in 95% ethyl alcohol is prepared. This is stored in a dark, cool cupboard and appears to keep for some months. For use a 1 in 5 dilution is made in glacial acetic acid. A faecal suspension, as for the benzidine test, is prepared. To 1 ml. of orthotolidine reagent are added 0·25 ml. of the faecal suspension and 0·25 ml. of hydrogen peroxide, 20 vol. strength, and mixed. The result is read after three minutes.

Strong positive.....	dark green
Moderate positive ...	medium green
Weak positive	pale green
Negative	no colour change

OCCULTEST METHOD A thin smear of faeces is made on the filter paper provided with the tablets. (It is stated that ordinary grades of filter paper may give false results.) An Occultest tablet is placed on the smear and one drop of distilled water placed on it. Five to 10 seconds later a second drop is added so that the water runs over the edge of the tablet on to the faecal smear. A reading is taken after two minutes.

Strong positive	deep blue colour extending widely beyond tablet
Moderate positive...	medium blue colour extending slightly beyond tablet
Weak positive	blue-green colour seen only when the tablet is removed
Negative.....	no colour change

TABLE I

SENSITIVITY OF TESTS ON DILUTIONS OF BLOOD IN WATER AND IN FAECES

Test	*Dilutions of Blood*					
	1/500	*1/2,500*	*1/12,500*	*1/62,500*	*1/312,500*	*1/1,562,500*
Dilutions of Blood—						
Benzidine	+++	+++	++	+	+	–
Occultest	+++	+++	++	++	+	–
Orthotolidine	+++	++	+	+	Trace	–
Hematest	+++	+++	++	+	Trace	–
Dilutions of Blood in Faeces						
Benzidine	+++	+++	++	+	+	–
Occultest	+++	++	+	Trace	–	–
Orthotolidine	+++	++	Trace	–	–	–
Hematest	+++	+	Trace	–	–	–

Strong positive +++ Moderate positive ++ Weak positive + Negative –

HEMATEST METHOD The method and interpretation are identical with those of the Occultest method, a Hematest tablet being substituted for an Occultest tablet.

SEPECTROSCOPIC EXAMINATION This is a semi-quantitative modification of the method described by Snapper (1919) for the demonstration of protoporphyrin, as the acid porphyrin, in faeces. About 30 g. of faeces is ground up in an excess (about 100 ml.) of acetone and filtered. The treated faeces are given a further wash with about 50 ml. of acetone and allowed to dry. Five grams of the dried faeces is ground up in 10 to 15 ml. of a mixture of one part of glacial acetic acid to two of ethyl acetate and filtered into a ½-in. test-tube and 8 to 10 ml. of filtrate is obtained. Then 4 ml. of 20% hydrochloric acid is added and the solutions mixed. Ether is added to 1 cm. from the top of the tube and the tube shaken. The mixture is allowed to separate, and the lower layer examined in a Hartridge reversion spectroscope with the aperture set at a reading of 10. The two absorption bands of acid protoporphyrin may be seen, a dense β band between the yellow and green (5650 – 5500A, $\lambda = 5572$ A) and a weaker α band between the red and yellow (6050 – 6000 A, $\lambda = 6024$ A), as described by Snapper (1919) and by Harrison (1947).

Strong positive..... both bands dense. The solution may require further dilution with acid for the bands to be defined in very strongly positive cases
Moderate positive ... β band strong, α band weak
Weak positive ..... β band weak, α band invisible
Negative ..... no band visible

Re-extraction of the dried faeces with ethyl acetate-acetic acid gives negative results in all but strongly positive specimens. Diagnostic errors through incomplete extraction do not therefore occur.

EXPERIMENTAL OBSERVATIONS

The sensitivities of the four chemical methods were compared against a series of fivefold dilutions of blood in distilled water and of blood in a 10% emulsion of faeces. The results are shown in Table I.

These tests were carried out on specimens of oxalated venous blood, haemoglobin 96% (14 g. per 100 ml.). Tests using a specimen with a haemoglobin of 64% (9·3 g. per 100 ml.) gave slightly weaker results.

To demonstrate the quantitative relationship between the various intensities of reaction in the test for porphyrin, a hydrochloric acid solution of porphyrin was made up from a positive specimen of faeces and a series of dilutions of this in a 20% solution of hydrochloric acid prepared and examined. The results are shown in Table II.

TABLE II

EFFECT OF DILUTION ON PORPHYRIN REACTION

Concentration of Original Acid Porphyrin Solution (%)	*Intensity of Spectroscopic Findings*	*Symbol*
100	Intense positive	+++
90	Very strong positive	
80	Strong positive	
70	Fairly strong positive	++
60	Moderate positive	
50	Rather weak positive	+
40	Weak positive	
30	Weak positive	
20	Trace	–
10	Negative	

Strong positive +++ Moderate positive ++
Weak positive + Negative –

Intensities greater than that shown by the "100%" solution in this case are possible and not at all uncommon.

SENSITIVITIES IN CLINICAL MATERIAL

All five tests were performed on three series of consecutive specimens, totalling 295 specimens, submitted to the laboratory, from in-patients and

out-patients, for routine investigation. The results are shown in Table III.

TABLE III

TESTS FOR OCCULT BLOOD AND PROTOPORPHYRIN IN CLINICAL MATERIAL STUDIED

Test	*Results*			
	Negative	+	++	+++
Benzidine	112	47	62	74
Occultest	132	55	48	60
Orthotolidine	181	28	27	59
Hematest	207	46	24	18
Protoporphyrin	68	49	85	93

Strong positive +++ Moderate positive ++ Weak +

RELATIONSHIP OF TESTS FOR OCCULT BLOOD AND PORPHYRIN TO CLINICAL FINDINGS

Three series of cases were analysed to determine the relationship of these tests to clinical diagnosis. In all instances the specimens were obtained after the patient had been for three days on a diet free of meat and green vegetables. The series of cases were:

(1) NORMAL CONTROLS These were 30 in-patients not known at the time of the test to have alimentary disease.

(2) TEST SERIES This was a consecutive series of 67 in-patients from whom specimens were submitted for routine examination to exclude bleeding.

(3) SELECT SERIES This was a selected series of 31 cases from our records to illustrate the lack of correlation between tests for occult blood and the test for porphyrin.

(1) NORMAL CONTROLS Of these, 20 gave negative results with all four chemical tests and with the test for porphyrin, They suffered from a variety of clinical conditions, none being associated with alimentary tract disease. Details of the remainder are given in Table IV.

(2) TEST SERIES This series can be divided into the following groups:

I. *Lesions of Upper Alimentary Canal* Of the 30 verified cases, there were 14 duodenal ulcers, 14 gastric ulcers, one anastomotic ulcer, and one gastric carcinoma.

All of the unverified cases (11) presented with clinical findings indicative of peptic ulceration and were treated as such. Four were not radiographed. One showed a hiatus hernia but ulceration was not verified. Six were negative on barium meal examination.

In four cases specimens were submitted to check on healing in known cases of peptic ulceration. In three of these, previous tests had been carried out, the porphyrin test in all three being then strongly positive while the benzidine reactions were negative, moderate positive, and strong positive respectively. In a fifth case, *x*-ray examination suggested a prepyloric ulcer, but tests for occult blood by all four methods and for porphyrin were negative. At operation the only lesion found was the scar of an old healed duodenal ulcer.

II. *Lesions of Lower Alimentary Canal* Of these there were three cases of haemorrhoids, one of diverticulitis of the lower colon, one of granuloma of the transverse colon, and one gastro-jejuno-colic fistula. The last is included here since bleeding would present as though coming from the colon.

III. *Congestive Cardiac Failure* Three of these

TABLE IV

'NORMAL' CONTROLS GIVING POSITIVE TESTS

Case	*Porphyrin*	*Benzidine*	*Occultest*	*O-Tolidine*	*Hematest*	*Clinical Details*
A.W.	–	+++	++	+++	+	Psychoneurosis, diarrhoea of unknown aetiology
	–	+++	++	+++	++	
A.D.	–	+++	+	++	–	Chronic bronchitis and emphysema, no apparent alimentary disease
A.H.	–	++	++	++	–	Fibrositis, very constipated, no apparent alimentary disease
M.S.	–	++	+	+	+	Breast abscess, no apparent alimentary disease
R.W.	+	+++	++	+++	+	Diverticulitis of colon
T.W.	+	+++	++	++	–	L. iliac colostomy, resection of carcinoma of colon 1 year before Haemorrhoidectomy 3 years before
V.M.	+++	+++	+++	+++	+	Clinical recurrence of duodenal ulcer proven in 1948 Barium meal negative
R.F.	+++	+++	+++	+++	–	Senility, hypertension, vomiting, Hb 29%, recticulocytes 2·7%. Unfit for further investigation
E.H.	+++	+	+	–	–	Coronary thrombosis and chronic gastric ulcer
M.S.	+++	–	–	–	–	Rheumatoid arthritis, epigastric tenderness, iron-deficiency anaemia

Strong positive +++ Moderate positive ++. Weak positive + Negative –

TABLE V

FAECAL FINDINGS IN TEST SERIES

Group	Diagnosis	Test	Results			
			0	+	++	+++
I	Active lesions of upper alimentary canal	Porphyrin	3*	3*	10	25
		Benzidine	7	5	14	15
		Occultest	11	11	7	12
		Orthotolidine	22	6	2	11
		Hematest	23	10	3	5
	Healed or healing lesions	Porphyrin	4	1	—	—
		Benzidine	2	1	1	1
		Occultest	2	1	2	—
		Orthotolidine	3	2	—	—
		Hematest	4	1	—	—
II	Lesion of lower alimentary canal	Porphyrin	5	1†	—	—
		Benzidine	—	—	1	5
		Occultest	—	—	4	2
		Orthotolidine	1	—	2	3
		Hematest	3	—	—	3
III	Congestive cardiac failure	Porphyrin	—	—	2	2
		Benzidine	—	—	—	4
		Occultest	—	2	—	2
		Orthotolidine	—	—	1	3
		Hematest	1	1	2	—
IV	No alimentary lesion	Porphyrin	3	3‡	—	—
		Benzidine	3	2	1‡	—
		Occultest	2	3	1‡	—
		Orthotolidine	5‡	1	—	—
		Hematest	4	2‡	—	—

Strong positive +++ Moderate positive ++ Weak positive + Negative −
*Two of each of these groups of three cases were on antacid absorbents at the time of test
†Granuloma of transverse colon
‡Case of myeloid leukaemia included

TABLE VI

RESULTS IN SELECT SERIES

Diagnosis	Benzidine Test				Protoporphyrin			
	0	+	++	+++	0	+	++	+++
Pyloric or duodenal ulcer (20 cases)	25	5	—	—	—	—	16	14
Gastric ulcer (9 cases)	12	1	—	—	—	—	6	7
Anastomic or jejunal ulcer (2 cases)	1	1	—	—	—	—	1	1

Strong positive +++ Moderate positive ++ Weak positive + Negative 0

cases were due to chronic bronchitis and emphysema, and one to mitral stenosis with auricular fibrillation. None gave evidence of alimentary disease.

IV. *Cases with No Alimentary Disease* Five of these were, respectively, anaemia without bleeding, lobar pneumonia, hypertension, hysteria, and mild chronic oesophagitis. The sixth was a case of myeloid leukaemia with skin petechiae, in which somewhat positive results were obtained.

V. *Incompletely Diagnosed Cases* In these the possibility of an alimentary lesion was overshadowed by the major condition present, namely, cerebral tumour, carcinoma of bladder, carcinomatosis of unknown origin, carcinoma of lung, and appendix abscess in a senile patient. Positive results were obtained in these cases but as adequate investigation of the alimentary tract was impracticable, these are excluded from the discussion. The results of this series are shown in Table V.

In the complete series of 67 cases, the number of tests for occult blood which were negative in the presence of a moderately or strongly positive test for porphyrin were: benzidine, 6; Occultest, 10; orthotolidine, 22; Hematest, 24.

(3) SELECT SERIES. This consisted of 45 tests on 31 cases. All patients were dieted for the test and all had lesions verified by radiography, gastroscopy, operation, or necropsy. They illustrate the occurrence of negative or weakly positive benzidine tests in cases of peptic ulceration and show the porphyrin findings in such cases. The results are shown in Table VI.

DISCUSSION

Since benzidine ceased to be available, workers who used it will now have changed to an alternative reagent and, in so doing, will no doubt have chosen a method of the same degree of sensitivity as the one to which they were accustomed. We have shown (Table I) that comparison of sensitivities against dilutions of blood in water can be very misleading, and that such comparisons should be made on dilutions of blood in a faecal emulsion or, better, by noting the results obtained by the methods under examination carried out in parallel on a consecutive series of clinical specimens. Against watery solutions of blood the benzidine test and the Occultest method are about equally sensitive, with the orthotolidine test and the Hematest method a little less sensitive. Faeces, however, appear to inhibit orthotolidine methods without apparently affecting the benzidine test, which thereby becomes much more sensitive, under these conditions, than the others. We have been unable to verify the inhibitory effect of faeces on benzidine reported by Needham and Simpson (1952), who used the Gregersen test. Tests on clinical material (Table III) verify the greater sensitivity of the benzidine method and show that, of the three orthotolidine tests described, the Occultest method is the most sensitive and the Hematest method the least.

It has been shown, in the case of the benzidine test by Gregersen (1919) and in the case of the orthotolidine test by Kohn and O'Kelly (1955), that by altering the proportion of reagents used one can produce a series of modifications ranging from the very sensitive to the extremely insensitive. One may therefore select, with either method, a test which suits one's own requirements. The majority of workers appear to require simplicity and the minimum of apparatus so that the test may be done in the out-patient department, in the ward, or in the family doctor's surgery, and such a degree of sensitivity as to produce the minimum of false positive results while the patient is on an ordinary diet (Hughes, 1952; Needham and Simpson, 1952; Smith, 1958).

Most workers of this opinion have used the simple slide benzidine test described by Gregersen (1919) in its original or some modified form. Analysis of the results of Needham and Simpson (1952) shows that only 40% of their cases with lesions of the upper alimentary canal gave acceptable positive results. A further 14% gave trace reactions only, and 46% gave negative results. Nevertheless they found an incidence of about 7% of unexplained positive results. Dahl-Iversen and Nissen (1930), who used a series of Gregersen's tests in each of their cases, found 7·3% persistently negative and 25·5% intermittently negative results in cases of carcinoma of the small and large intestine.

Others have used the guaiac test to allow the test to be carried out without dietary restrictions. Hoerr, Bliss, and Kauffman (1949) using it found that over 20% of their negative results were obtained in cases in which blood would be expected in the faeces, including cases of carcinoma of the stomach, of the oesophagus, and of the rectum. Nevertheless they found eight unexplainable positive results out of 39. With the guaiac test, Mason and Belfus (1952) found that 43% of their positive results occurred in patients with, and 48% in patients without, alimentary lesions. The remainder were doubtful or the patients failed to cooperate. Of their negative results, half occurred in patients with gastrointestinal symptoms and 20% of the total in patients with proven alimentary lesions, including cases of gastric carcinoma. Therefore we must consider whether simplicity of operation and no dieting before the test, at the same time achieving no false positives, are the ideal requirements of a test for occult blood.

The means available to the clinician, other than clinical observation, for the diagnosis of peptic ulceration and gastric carcinoma are, generally speaking, exploratory laparatomy, *x*-ray examination, gastroscopy, and laboratory methods, of which the faecal occult blood test is the most commonly used. Exploratory laparatomy is not often resorted to in the absence of other supporting evidence and cannot possibly be carried out as a diagnostic procedure in every vaguely suspect case. With regard to *x*-ray examinations, Tanner (1944) found that of 631 gastric ulcers seen at gastroscopy, 159 (25%) were not found on *x*-ray examination. In two cases the radiological diagnosis was duodenal ulcer and the final diagnosis gastric carcinoma. Thirteen cases of gastric carcinoma were thought to be benign ulcers or not seen at all at *x*-ray examination. He points out also that the radiological diagnosis of anastomotic ulcers is notably difficult. Schindler (1952) found that of 70 gastric ulcers, 15 (21%) were not seen on *x*-ray examination. In certain instances highly experienced radiologists failed to demonstrate ulcers although they knew in advance that they existed and exactly where.

Similarly in the series of Needham and Simpson (1952), of 213 cases of duodenal ulcer, 62 (29%) were not found at *x*-ray examination.

With regard to gastroscopy, Schindler reports that 26·5% of gastric ulcers were not seen by that method and, further, that 7·7% of malignant ulcers of stomach were not diagnosed either by *x*-ray or gastroscopic examination.

We agree, therefore, with Rider and Owens (1954) that 'one may be lulled into a false sense of security by false negative reactions if the occult blood test is insensitive.' They used a sensitive benzidine test and adequate dieting and obtained only 1% false positive results. Gurling (1951) obtained 10% false positive results with a similarly sensitive method. We do not agree with Hughes (1952) that Gurling's result 'detract markedly from the clinical significance of a positive result' and stress that at least as important is the clinical significance of a negative result. We note also the high incidence of unexplainable positive results by both Gregersen's and the guaiac tests in undieted patients.

It appears that, while other methods of diagnosis are so uncertain, the clinician should expect more help than is offered by simple insensitive methods of testing for occult blood. With these, half of their cases of alimentary bleeding give negative results, and, on the other hand, of all the negative results, half occur in cases of alimentary bleeding. In addition, they appear to give a high proportion of unexplainable positive results. A test for occult blood should be within the scope of the routine hospital laboratory, but it is allowable that it requires some apparatus, some skill, and some care. It should detect blood when it is present, which means that if the test is to be sufficiently sensitive the patient must be dieted beforehand, and the doctor requiring the test should be aware of the substances which may interfere with it (Bramkamp, 1929; Johnson and Oliver, 1941; Peranio and Bruger, 1951; Hughes, 1952; Needham and Simpson, 1952; Kohn and O'Kelly, 1955) and ensure that they are not allowed to do so. In addition the test should, if possible, give some indication of the level in the alimentary canal at which bleeding is taking place.

Bramkamp (1929) pointed out that 90 times as much blood requires to be given by mouth as to be added directly to a stool to give a positive test for occult blood. We have drawn attention to the incidence of negative tests in the presence of alimentary bleeding, particularly in the case of the upper alimentary tract, and have observed this even with highly sensitive methods (Table VI). The reason for these findings is that haemoglobin on its way down the alimentary canal is converted to protoporphyrin, which gives a negative test for occult blood.

The technique of Snapper (1919) was designed to demonstrate both unaltered blood (haematin) and blood converted to protoporphyrin. We have chosen his technique for the demonstration of protoporphyrin, as the acid porphyrin, and have increased its sensitivity about four or five times. It will be seen (Table II) that this increase in sensitivity would convert false negative and trace reactions into moderate and strongly positive tests. By making the test semiquantitative, and standardizing the method of examination, we have achieved some degree of reproducibility of results. We have not determined the sensitivity of this method in terms of blood loss, but note that Bloem (1933) was able to demonstrate acid porphyrin in faeces in a concentration equivalent to 140 mg. haemoglobin per 100 g. moist faeces, or about 1 to 2 ml. of blood in an ordinary stool. That Snapper's method is insufficiently sensitive is shown by the results obtained by Gurling (1951). Combining the complete Snapper's test with a benzidine test he found an incidence of 52% false negatives in cases of peptic ulcer, and of 18% in cases of gastric carcinoma.

The incidence of strongly positive tests for porphyrin in association with negative occult blood tests is high and, as has been noted, the less sensitive the test for occult blood, the greater this incidence. Such results occur almost invariably with lesions of the upper alimentary canal. Contrary to the findings of Hughes (1952) we have observed many instances of anaemia secondary to alimentary bleeding in which even a sensitive benzidine test has been negative. In such cases positive tests for porphyrin have been of great assistance. We have also pointed out, in connexion with experiments on series of dilutions of blood, that in the presence of anaemia the tests for occult blood become less sensitive, for under these circumstances the loss of haemoglobin into the alimentary canal is correspondingly less.

In persons without alimentary lesions tests for occult blood and for porphyrin should be negative. Of our 30 controls, 20 gave such negative results. In the other 10 (Table IV) there was no case in which some explanation could not be offered for the presence of porphyrin and in only two was there no explanation of positive chemical tests.

In the test series (Table V) certain points of importance are illustrated. In active lesions of the upper alimentary tract, six only gave negative or weakly positive tests for porphyrin, and four of these occurred in patients on antacids. Ryffel and Payne (1923) similarly observed that the test for porphyrin becomes negative when the Sippy method of treatment, incorporating the administration of large doses of antacid, is instituted. Charcoal and certain

antacids. particularly the salts of bismuth, aluminium, and magnesium, strongly adsorb protoporphyrin and may render this test negative. If the antacid is withdrawn the test becomes positive.

We have also noted that sometimes after a massive haemorrhage the test for porphyrin may become negative while the benzidine test is intensely positive. This lasts for a day or two, and porphyrin rapidly appears in high concentration. One of the remaining two weak results was of this type. In the other we can offer no explanation other than that the patient just was not bleeding at that time.

In healing lesions the test for porphyrin appears to be of value. In lesions of the colon and rectum tests for porphyrin are negative or weakly positive, as would be expected, while chemical tests, provided they are of sufficient sensitivity, are positive (Table V). We have, however, seen a number of cases of carcinoma of the caecum and upper colon in which the test for porphyrin was strongly positive as well as the benzidine test. This exception apart we find that a strongly positive porphyrin test means upper alimentary bleeding.

Congestive cardiac failure almost always gives positive tests for occult blood and porphyrin. The passive congestion of the alimentary mucosa in these cases is a sufficient explanation of the positive findings.

In this series patients with no alimentary lesion gave negative or weakly positive results only, except in a patient with myeloid leukaemia with skin petechiae, in whom positive results would be expected.

We have pointed out that our patients are normally on a diet free of meat and green vegetables before the test, and that we consider an absence of meat of importance in relation to the test for blood. We believe also that it is of importance in connexion with the test for porphyrin, although our experience is at variance with that of Ryffel and Payne (1923) who state that the spectrum of protoporphyrin ('haematoporphyrin') is not found in the faeces following the ingestion of meat. In addition the close similarity of the structure of chlorophyll to that of haemoglobin indicates that it also should be omitted from the diet in a test for porphyrin.

It is possible that certain diseases associated with abnormal porphyrin excretion without alimentary bleeding, such as pernicious anaemia and certain types of porphyria, may cause confusion but do not present a great problem although they should be kept in mind.

In conclusion, we are of the opinion that the test for faecal occult blood should be carried out by a sensitive method and be accompanied by a sensitive test for protoporphyrin. The Hematest method is too insensitive to be of real value and the orthotolidine tube test we describe is little better. The Occultest method is the most sensitive of these three and, in our hands, has about the same degree of sensitivity as Gregersen's slide method, for which it would be a satisfactory and very simple substitute. Also, adequate precautions should be taken regarding diet and drugs given before the test. False positives will inevitably occur but, under these circumstances, many of them may be due to the impossibility of otherwise demonstrating a lesion rather than to its absence.

SUMMARY

Comparative observations are made on a benzidine test, an orthotolidine test, the Occultest method, and the Hematest method for the detection of occult blood in faeces. The importance of carrying out such comparisons of faecal rather than on watery suspensions of blood is stressed.

A sensitive test for protoporphyrin is described and its value as an adjunct to tests for occult blood discussed. The importance of using sensitive rather than insensitive tests is illustrated, and the necessity to diet the patient before the test and to withdraw interfering substances in the form of medicines discussed.

Our thanks are due to the Ames Company (London), Limited, for generous supplies of Occultest and Hematest reagent tablets, and to the clinical staff of St. Andrew's Hospital who provided the clinical material on which this study is based.

REFERENCES

Bloem, T. F. (1933). *Biochem. J.*, **27**, 121-131.
Bramkamp, R. G. (1929). *J. Lab. clin. Med.*, **14**, 1087-1091.
Case, R. A. M., Hosker, M. E., Drever, M.McD., and Pearson, J. T. (1954). *Brit. J. industr. Med.*, **11**, 75-104.
Dahl-Iversen, E., and Nissen, N. I. (1930). *Hospitalstidende*, **73**, 543-554. Quoted in *J. Amer. med. Ass.* (1930), **95**, 702.
Gregerson, J. P. (1919). *Arch. Verdau.-Kr.*, **25**, 169-195.
Gurling, K. J. (1951). *Postgrad. med. J.*, **27**, 345-348.
Harrison, G. A. (1947). *Chemical Methods in Clinical Medicine*, 3rd ed., p. 310. Churchill, London.
Hoerr, S. O., Bliss, W. R., and Kauffman, J. (1949). *J. Amer. med. Ass.*, **141**, 1213-1217.
Hughes, A. (1952). *Brit. med. J.*, **2**, 970-975.
Johnson, A. S., and Oliver, E. B. (1941). *J. Lab. clin. Med.*, **26**, 727-728.
Kohn, J., and O'Kelly, T. (1955). *J. clin. Path.*, **8**, 249-251.
Mason, E. W., and Belfus, F. H. (1952). *J. Amer. med. Ass.*, **149**, 1526-1528.
Needham, C. D., and Simpson, R. G. (1952). *Quart. J. Med.*, **21**, 123-133.
Peranio, A., and Bruger, M. (1951). *J. Lab. clin. Med.*, **38**, 433-445.
Rider, J. A., and Owens, F. J. (1954). *J. Amer. med. Ass.*, **156**, 31-33.
Ryffel, J. H., and Payne, W. W. (1923). *Guy's Hosp. Rep.*, **73**. (4 ser. 3), 131-136.
Schindler, R. (1952). *Med. Clin. N. Amer.*, **36**, 1099-1110.
Smith, R. L. (1958). *Brit. med. J.*, **1**, 1336-1339.
Snapper, J. (1919). *Arch. Verdau.-Kr.*, **25**, 230-240.
Tanner, N. C. (1944). *Brit. med. J.*, **2**, 849-851.

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The Nile blue test in the detection of steatorrhoea

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SYNOPSIS The Nile blue test for the detection of steatorrhoea has been compared with the estimation of 24-hour faecal excretion by the method of Weijers and Van der Kamer modified by Anderson, Frazer, French, Gerrard, Simmons, and Smellie (1952). The Nile blue test is easily and quickly carried out. A positive result was only found in cases showing a considerable degree of steatorrhoea but it was often negative when the excretion of fat was abnormally high. This test appears to be of considerable use as a rapid, semiquantitative screening test in the investigation of possible cases of steatorrhoea.

The usual laboratory methods for the estimation of fat in faeces are accurate but extremely time consuming. In Great Britain the method most used is that of Weijers and Van der Kamer, as modified by Anderson, *et al.* (1952). In this method the output of fat in 24 hours is estimated by analysis of an aliquot of faeces collected over a known period. This is almost certainly the most accurate method available, but takes two and a half to three hours for each estimation, and there is no means of 'bulking' the tests, so that a number may be carried out simultaneously. Consequently, to carry out a number of such estimations throws a heavy burden on any laboratory. The same objections apply to other clinical methods, and therefore the estimation of the stools for fat is probably not undertaken as often as it should be.

A number of ways have been suggested as qualitative rather than quantitative tests for excess faecal fat. The naked-eye inspection of the stools is often enough for diagnosis, but there are cases in which the appearance is remarkably normal even though the fat content may be high. Microscopic examination of the stools for fat crystals and fat droplets has been used extensively in the past as a rapid screening test for excessive fat but this is very inaccurate, as has been shown by Demole and Thommen (1949).

For these reasons a rapid, semi-quantitative test for faecal fat would be of great clinical value for the diagnosis of steatorrhoea. Demole and Howard (1958) have applied the Nile blue reaction as a test on these lines. This is based on the principle that Nile blue in the presence of sodium carbonate gives a blue colour with oleates, and the depth of this colour will be proportionate to the amount of oleates present. These authors compared this test with quantitative estimations of the percentage of fat in the faeces, as estimated by the modified (Anderson *et al.*, 1952) method of Weijers and Van der Kamer. They found a good correlation between the tests. The percentage of fat in the faeces, however, is not usually held to be the best guide to the pathological quantities of fat being excreted, and it seemed that it would be worth while attempting to correlate the Nile blue test with the 24-hour faecal fat excretion.

METHOD

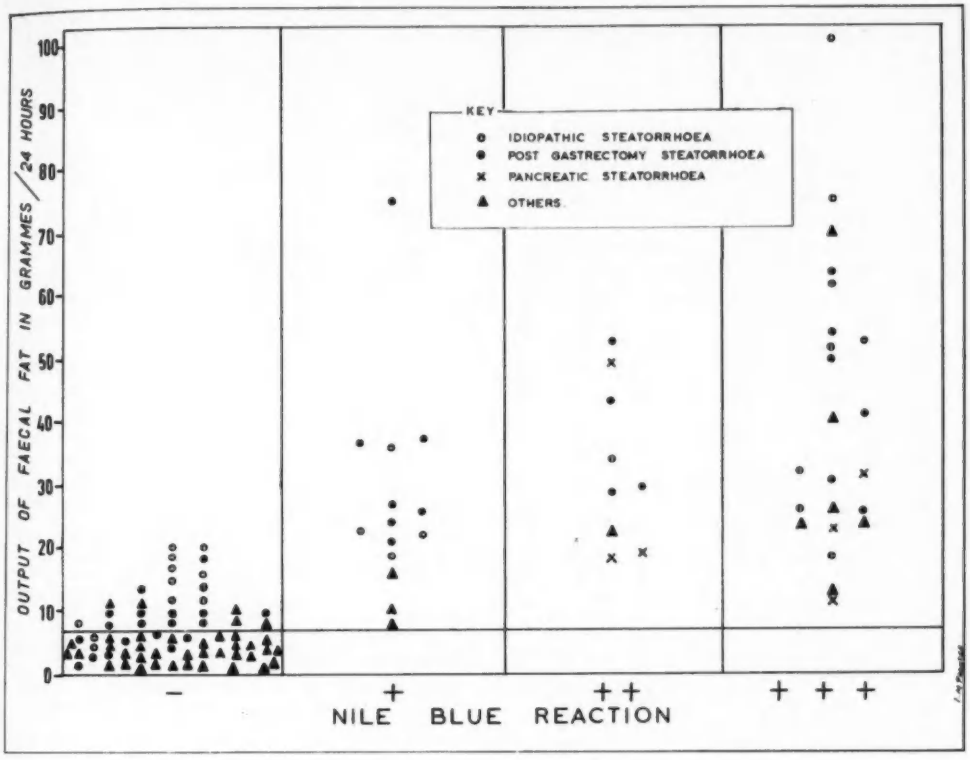
The method used for the Nile blue reaction was the same as that described by Demole and Howard (1958), and was originally described by Goiffon (1948).

REAGENTS
- 0·05% Nile blue
- 20% pure sodium carbonate
- 10% hydrochloric acid
- Saturated aqueous solution of ammonium oxalate

PROCEDURE

Faeces to be examined are diluted to a concentration of approximately 10%, and mixed well. To 1 ml. of this dilute faecal suspension is added in a test tube 1 ml. of distilled water, 2 drops of 10% Hcl., and 1 drop of the saturated solution of ammonium oxalate. The mixture is heated to boiling for a few seconds, then cooled and poured into a 25 ml. beaker. The test tube is washed with 2·5 ml. of sodium carbonate, and the washings poured into the beaker until the total volume is 20 ml., when 1 ml. of Nile blue is added.

A single estimation takes 15-20 minutes, and a number of estimations can be carried out simultaneously. The colour of the mixture is read immediately. The colours range from pink-grey (O) through grey blue (+) light blue (++) to deep blue (+++). The total faecal fat



in 24 hours was estimated by the modified method of Weijers and Van der Kamer (Anderson *et al.*, 1952).

The faeces investigated were an unselected series of specimens received in the laboratory for faecal fat estimation and were from patients with a variety of clinical conditions.

One hundred and sixteen specimens were examined from some 80 patients. Each specimen consisted of a three-day collection of faeces from patients on normal hospital diet.

RESULTS AND DISCUSSION

The results are shown in the table and the figure.

In every case in which the Nile blue test was positive there was more than 7 g. of fat in the 24-hour stool. On the other hand, the test was frequently negative when the 24-hour output was pathologically high. In two cases a 24-hour output of nearly 20 g. gave a normal Nile blue test. Although the ranges in each group are great, the colour reaction increases in parallel with the 24-hour faecal fat. It will be noted that one case, giving only a 1+

TABLE

RESULTS OF FAECAL FAT ESTIMATION BY MEANS OF NILE BLUE REACTION COMPARED WITH RESULTS OF CHEMICAL ANALYSIS

No. of Examinations	*Nile Blue Reaction*	*Chemical Reaction Fat Output (g./24 hr.)*		
		Minimum	*Average*	*Maximum*
70	—	0·57	6·7	19·8
14	+	7·1	26·39	74·8
9	++	17·8	32·47	52·3
23	+++	11·17	40·58	113·7

Nile blue reaction had a faecal fat content of 75 g./24 hours. This specimen was analysed by gas chromatography and the percentage of oleates was unusually low, accounting for only 9% of the total fatty acids. This is a lower percentage than has been encountered in any other case investigated, and appears to confirm that it is the oleates that are responsible for the Nile blue reaction.

There would appear to be little relationship between the type of disease producing the steatorrhoea and the results of the tests. The cases of pancreatic

steatorrhoea do show a rather strong Nile blue reaction proportionate to the amount of the 24-hour faecal fat, but only six cases of this type of steatorrhoea were investigated and this may well be a chance relationship rather than an indication that there are relatively more oleates in the faecal fat of these patients than in that of those suffering from other types of steatorrhoea.

The results suggest that a positive Nile blue test is indicative of a pathological degree of steatorrhoea, and as the time taken for this test is only a fraction of that needed for quantitative chemical analysis by the accepted methods, this test could prove a useful screening test in the investigation of cases of diarrhoea and anaemia. It does not, however, in its present form offer a means of excluding such a diagnosis, as a considerable degree of steatorrhoea (as assessed by a 24-hour faecal fat) may be associated with a negative Nile blue test.

REFERENCES

Anderson, C. M., Frazer, A. C., French, J. M., Gerrard, J. W., Simmons, H. G., and Smellie, J. M. (1952). *Lancet*, **1**, 836-842.
Demole, M. J., and Howard, E. M. (1958). *Amer. J. dig. Dis.*, n.s.**3**, 549-556.
——, and Thommen, B. (1949). *Arch. Mal. Appar. dig.*, **38**, 1128-1132.
Goiffon, R. (1948). *Ann. Biol. clin.*, **6**, 282.

The December 1960 Issue

THE DECEMBER 1960 ISSUE CONTAINS THE FOLLOWING PAPERS

Heredity in Gastroenterology: A Review R. B. MCCONNELL

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Gastroenterological Society of Australia

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The annual meeting of the British Society of Gastroenterology

The annual meeting of the British Society of Gastroenterology was held at King's College Hospital, London, on 4 and 5 November 1960 under the Presidency of Mr. Harold Edwards, with Dr. Bruce Pearson as the local secretary.

At the general business meeting the following elections were made: President, E. Bulmer; Treasurer, Hermon Taylor; Secretary, W. A. Bourne; Council Members, S. Sherlock and S. C. Truelove. The following were elected full members of the Society: Michael Atkinson, D. H. Cummack, David Douglas, C. W. A. Falconer, A. P. M. Forrest, Terence Fulton, J. B. Gibson, R. H. Girdwood, R. A. MacMynn, J. G. Murray, W. A. B. Nicholson, Brian Schofield, Geoffrey Slaney, and Ian Todd. The following were elected as associate members: E. D. Acheson, H. T. F. Barniville, E. V. Cox, C. W. Crane, G. P. Crean, I. E. Gillespie, F. E. Higgins, I. D. A. Johnston, W. G. Manderson, G. F. Matts, A. G. Melrose, Annette Rawson, H. I. Tankel, and R. S. Williams.

It was decided that the Society should play a more active role in international relations in respect of gastroenterological work and that there was a need to keep in touch with other international societies and to assist in the coordination of education and research activities. It was decided to form a special committee with T. C. Hunt, F. Avery Jones, Hermon Taylor, and Geoffrey Watkinson as members.

On the Friday evening a most excellent dinner was held at the Apothecaries Hall and this had been arranged by Professor Magnus. The President spoke of the work of the Society and welcomed the two visitors, Dr. Grossman and Sir William Morrow.

After dinner the Clerk of the Apothecaries gave an account of the history of the beautiful hall.

Scientific meeting

THE EFFECT OF PROSTIGMINE ON HUMAN COLONIC MOTILITY

N. A. CHAUDHARY AND S. C. TRUELOVE (OXFORD) had made a comparative study of the patterns of colonic motility following a standard intramuscular dose of prostigmine methylsulphate in normal subjects, patients with irritable colon syndrome, and patients with ulcerative colitis. In normal subjects, the standard dose of prostigmine methylsulphate provoked increased activity with a resulting pattern not dissimilar from that of the resting pattern of patients with spastic colon in a stage of symptoms. Patients in all clinical categories of the irritable colon syndrome displayed more colonic activity after prostigmine than normal subjects. This was so whether or not they were in a stage of symptoms. By contrast, the response of patients with ulcerative colitis to a standard dose of prostigmine was little if any different from that of normal subjects. These results suggest that patients with spastic colon are subject to excessive parasympathetic stimulation or to increased reactivity to normal stimuli.

RADIOLOGICAL AND MANOMETRIC STUDIES OF CHANGES IN MOTILITY OF THE ALIMENTARY TRACT IN DIFFUSE SYSTEMIC SCLEROSIS AND POLYMYOSITIS

D. A. W. EDWARDS (LONDON) said that in a group of 21 patients with scleroderma, dermatomyositis, polymyositis, or systemic sclerosis, the motor activity of the gut had been examined by radiology and by recording changes in intraluminal pressure with open tubes and miniature balloons. The majority of patients were investigated personally; information about the others was obtained from hospital records. In all but two patients there was evidence of a marked decrease in smooth muscle activity in some part of the gut, and, in a small group in which histological material became available, a decrease in the amount of smooth muscle was demonstrated.

The commonest disturbance was loss of oesophageal peristalsis, sometimes associated with stiffening of the lower end of the oesophagus and loss of tone in the cardiac sphincter. Changes in motility of the stomach were difficult to distinguish from the normal, but the characteristic squashed out atonic immotile duodenal loop was present in six out of 13 patients adequately examined. A similar appearance of flattened immotile loops of small intestine was seen in five out of 10 patients. Five out of six patients had saccular dilatations and a disorganized haustral pattern in the colon.

The characteristic changes are valuable as a diagnostic aid when biochemical or external manifestations of this disease complex are absent or equivocal, or when the disease presents as a gastroenterological problem. Loss of smooth muscle appears to be an integral part of the disease process.

GASTRIC SECRETION IN LIVER DISEASE

SOAD TABAQCHALI AND A. M. DAWSON (LONDON) It has been claimed that there is a high incidence of peptic

ulcer in patients with portal cirrhosis. Recently, it has been suggested that this might be related to the fact that in dogs which have undergone a portal systemic shunt procedure there is a consistent increase in acid secretion from a Heidenhain pouch.

The records of 200 consecutive patients with portal cirrhosis were analysed and a probable increased incidence of peptic ulcer, especially in women, was found, in contrast to the greatly increased incidence in patients with primary biliary cirrhosis. Gastric secretory function was assessed in patients with portal cirrhosis, some of whom had had a portacaval anastomosis operation, and in patients with primary biliary cirrhosis. This was done by measuring basal acid secretion, maximal histamine secretion, and the 24-hourly gastric *p*H. In all groups the maximal response to histamine was normal. However, in patients with a portacaval anastomosis there was a tendency to a low gastric *p*H at night, whereas patients with uncomplicated portal cirrhosis and primary biliary cirrhosis did not differ from the controls in this respect.

A POSSIBLE EXPLANATION OF FAILURE OF RADIO-TRIOLEIN TO DIAGNOSE STEATORRHOEA

A G. COX (SHEFFIELD) In a clinical trial, radio-iodinated triolein failed to diagnose steatorrhoea in 50% of cases with a high chemical faecal fat excretion. A possible explanation was instability of the radio-iodine label in the alimentary tract. To investigate this point, a method using an anion exchange resin was evolved and applied *in vitro*. When incubated with duodenal juice, six batches of triolein tagged with the standard I^{131} Cl. label showed a steady rise in the amount of the radio-isotope liberated. This demonstration of the instability of the label requires study *in vivo*. The method also confirmed that the simple I^{131} label, still occasionally used, is highly unstable.

In the discussion N. Coghill noted that Lubran, working with him, had obtained good correlation and it was agreed that results varied appreciably in different places. Cooke pointed out that strictly speaking steatorrhoea was demonstrated and not diagnosed.

OBSERVATIONS ON THE CLINICAL TESTING OF GASTRIC ANTISECRETORY DRUGS AND ANTACIDS

J. E. LENNARD-JONES (LONDON) had studied the effect of two gastric antisecretory drugs, a long-acting form of propantheline bromide and poldine methosulphate (Nacton), on the *p*H of hourly samples of gastric contents in patients with uncomplicated duodenal ulcer. Both the drugs significantly reduced the acidity of the sample when patients took hourly drinks of milk-cream without other food. When the same patients took a gastric-type diet, a small reduction in acidity was demonstrable with propantheline but no reduction with Nacton. In other patients taking a diet, Nacton given alone did not reduce acidity even when given in doses large enough to cause unpleasant side-effects. Nacton potentiated the effect of suboptimal doses of alkali and the combination reduced acidity during waking hours. Although Nacton delayed gastric emptying at night, the effect of a dose of alkali at bed-time was not prolonged. When patients sucked antacid tablets continuously a satisfactory reduction in acidity of the gastric contents during waking hours was achieved without the use of an antisecretory drug.

From this work it is concluded that the two antisecretory drugs studied are less effective in reducing the acidity of gastric contents under the conditions of clinical use than under experimental conditions. Antacids, sucked as tablets, satisfactorily reduce gastric acidity during the day; the two antisecretory drugs do not usefully reduce the *p*H of the gastric contents at night when a patient is taking a gastric-type diet.

In the discussion J. N. Hunt noted that the antisecretory drugs had been given for a relatively short time, about five days before the observations were made, and suggested that longer prior administration might have produced a different result. The speaker did not support this view.

Sircus commented on the complexity of the factors resulting in the final sampling figure.

SYMPOSIUM ON APPLIED PHYSIOLOGY OF THE STOMACH

There were four opening papers and these were given by Professor R. A. Gregory, J. G. Murray, M. R. Vane, and W. I. Card.

THE PHYSIOLOGY OF GASTRIC SECRETION

PROFESSOR R. A. GREGORY (LIVERPOOL) Studies during the last 10 years by Uvnas, Dragsted, Schofield, Gregory, and Tracy and others have led to a general acceptance of the suggestion first made by Straaten that vagal activity is important for the release of gastrin from the pyloric antrum. It is now agreed that in addition to its direct action on the gastric glands the vagus also excites the antrum and thus contributes, in combination with local stimulation by the swallowed food, to release the pyloric hormone. The 'cephalic phase', defined originally by Pavlov, thus involves a hormonal component. How long the release of gastrin continues is uncertain, but two important controlling factors are now recognized to exist. One is the intragastric accumulation of acid which inhibits further release of gastrin by a local action on the pyloric mucosa. The other, as recently shown by Gregory and Tracy, is the action of the hormone enterogastrone liberated from the duodenum by such agencies as fat, acidity, and hypertonicity of the duodenal contents.

In addition to this hormonal mechanism, reflex control of gastric secretion from the duodenum undoubtedly exists, and the vagi are probably the efferent pathway, as

indicated by the studies of Code and Watkinson. The highly purified preparation of gastrin obtained by Gregory and Tracy stimulates acid but not pepsin secretion in the conscious human subject, as well as in animals. Its action resembles that of histamine, but differs from this substance in that it is strongly inhibited by atropine, and single intravenous injections are effective in exciting secretion.

NERVOUS CONTROL OF GASTRIC FUNCTION

J. G. MURRAY (LONDON) Recent experimental work has thrown new light on the composition of the vagus nerve. About 80 to 90% of the fibres supplying the abdominal viscera are afferent, some arising from stretch receptors and others from chemoreceptors in the stomach.

Combined anatomical and physiological observations are consistent with the view that the intrinsic nerve plexuses contain local reflex arcs and mediate, for example, peristalsis. At least two sets of enteric neurons are involved: (*a*) sensory neurons, the processes of which are distributed to the mucous membrane on the one hand, and to motor neurons on the other, and (*b*) motor neurons which innervate the muscle coats. Of all the nervous tissue in the stomach and gut, by far the largest part is of intrinsic origin.

Lastly, the surprising plasticity of the autonomic nervous system following injury or disease has only been appreciated over the last few years. If an organ is partially denervated, then the remaining intact nerve fibres are stimulated to give off tiny branches. These sprouts connect up with denervated effector organs and result in return of function. Even after complete denervation, nearby nerve fibres may sprout to produce quite bizarre effects. This phenomenon was briefly described with particular reference to vagotomy.

THE PHARMACOLOGY OF THE SMOOTH MUSCLE OF THE STOMACH

J. R. VANE (LONDON) Dale and Feldberg in 1934 demonstrated that excitation of the vagus nerves brought about the release of acetylcholine in the wall of the stomach. However, the suggestion has often been made that vagal effects in the stomach are mediated by the release of histamine. This suggestion was based on the high potency of histamine as a stimulus for gastric secretion and for muscular contraction. Moreover, histamine is normally present in the stomach wall. More recently, another hormone, 5-hydroxytryptamine, had been isolated from the stomach. In consequence, the roles of acetylcholine, histamine, and 5-hydroxytryptamine have been re-investigated.

The release of acetylcholine from parasympathetic nerve endings has been confirmed. It has also been shown that the major effect of histamine on the smooth muscle of the stomach, which is to increase its tone, is brought about through an excitation of nerves.

CLINICAL APPLICATION

W. I. CARD (EDINBURGH) underlined the ideas found valuable in gastric physiology of the last 10 years. The most important general advance in the last 10 years has been the increasing application of the scientific method to gastroenterology, and much of this advance has been due to the introduction of better investigative techniques.

With the introduction of the electron microscope morphology has come into its own again, revealing almost a new dimension of thought. The obstacle here seems to be not that of resolution but of finding methods of differentiating structure.

Gastric secretion continues to command much of our attention, partly because it is simplest to measure and partly because high secretions play a part in the causation of certain peptic ulcers. The evidence that histamine is the actual pharmacological stimulant of the parietal cells continues to accumulate. The problem that has teased the physiologist for so long of inferring from the mixed gastric secretion the original constituents as secreted continues to appear insoluble. Over the last 20 years acid output rather than acid concentration in gastric juice has been generally adopted. Though the output is reproducible with the ordinary doses of histamine, the use of 'maximal' histamine stimulation does allow comparisons of one subject with another by excluding variations in one parameter, and also makes for a more rigorous definition of achlorhydria. The output of acid in a given time can be considered as the product of the rate of secretion and the number of secretory units, though the phrase 'rate of secretion' covers a number of factors. One of these which has been frequently overlooked is the blood flow to the mucosa which must, from consideration of energetics, be very large. Directly the acid output is shown to be related to the number of cells, the question immediately presents itself of accounting for the origin of a large cell mass. There seems to be a need to consider the existence and the role of trophic hormones, though it has not been possible to confirm the trophic action of histamine as had been previously reported. The possibility has to be considered that gastrin acts as a trophic hormone. The discovery of a gastrin-like hormone in the Zollinger-Ellison syndrome raised the question of whether this exists as a qualitatively distinct syndrome, or whether it is simply a grossly exaggerated form of a normal mechanism. Another hormone with a trophic action that had to be considered is cortisone. It has been shown that complete achlorhydria occurs in Addison's disease, that this may disappear after treatment with corticoids, and that even peptic ulceration may follow. Cortisone, given in an acute experiment, did not seem to produce an increased response to histamine in human subjects, but given over a longer period, as in the treatment of Crohn's disease, it undoubtedly increases the output of acid. This can only be due to an increase in the number of secretory units per cell, an increased number of cells, or an augmentation of secretory rate. The pituitary might also produce a trophic hormone from the gastric mucosa. There was atrophy in clinical hypopituitarism, a diminution of the parietal cell count had been demonstrated following experimental hypophysectomy, while the prolonged administration of corticotrophin in man produced increasing amounts of acid which it was difficult to believe could be due to anything else except hyperplasia of the parietal cells.

DISCUSSION

Professor A. W. Kay commented that Mr. Murray's fascinating studies on the intimate neuroanatomy of the stomach had an obvious bearing on the operation of surgical vagotomy. His demonstration of 'sprouting' from the divided ends of the vagal fibres rather suggested an appreciable chance of vagal regeneration with a risk of late recurrent ulceration. In this connexion it was of interest that secretion studies had been recently performed in Sheffield on patients who had undergone vagotomy from a few months to some years previously. Analysis of these results showed that the great majority showed no tendency to a rising level of acid secretion and indeed there was a general trend towards a further reduction.

With regard to Professor Gregory's magnificent survey of the physiology of gastric secretion, we must have some concern about various discrepancies between the results of animal and human investigations. For example, antrectomy in the dog has been shown by both American and Scandinavian investigators to have no effect on histamine-provoked acid secretion. On the contrary, antrectomy in man causes a marked reduction not only of histamine secretion but of maximally stimulated acid secretion. It may be that we should give more attention to physiological studies in primates and there is certainly room for a great deal more investigation of patients undergoing planned surgery for duodenal ulcer.

The influence of the advances in physiology and pharmacology on surgical treatment demands that careful preoperative studies on gastric secretion should be carried out and thereby the operation selected most suited to the individual case. In gastrectomy, vagotomy with a drainage procedure, antrectomy with vagotomy, and gastrojejunostomy we have four operations of value in surgical therapy. Our task is to devise methods from which we can select the appropriate operation for each patient.

J. N. Hunt drew attention to the threshold for the effect of acid in reducing gastric secretion. Equal concentrations of hydrochloric acid, acetic acid, and citric acid were about equipotent, although the *p*H of the solutions were quite different.

Professor R. B. Welbourn referred to the influence of the major endocrine glands on gastric acid secretion, which Dr. Card mentioned. Hypophysectomy reduced secretion profoundly in the rat. Corticotrophin and growth hormone restored it. The latter observation does not appear to have been taken up by others. Pharmacological doses of cortisone, methyl prednisolone (a 'pure' glucocorticoid), and aldosterone all increase secretion similarly in the dog. The effect might be a mineral corticoid one, since methyl prednisolone has slight salt-retaining properties. However, they had recently investigated triamcinolone and dexamethasone, of which the mineralocorticoids were minimal, and had found that they also increased secretion. It seems likely, therefore, that the gastric activity of the adrenal steroids is not necessarily tied to their main metabolic actions. Cortisone increased by about 50% the maximal secretory capacity, in response to histamine, of Heidenhain pouches in dogs and it increased the parietal cell mass by a similar proportion. The physiological role of all these hormones appears to be 'permissive'.

M. I. Grossman said that the release of gastrin by vagal impulses had been unequivocally established. Schofield had placed the keystone in the arch of evidence supporting this conclusion. We should not, however, lose sight of the fact that vagal impulses stimulate the secretion of gastric acid by another mechanism, namely, direct cholinergic action on the acid-secreting glands. It is not yet possible to say which of these two vagal mechanisms for stimulation of acid secretion is quantitatively predominant.

Professor Gregory had introduced a new concept of the mode of action of enterogastrone. He believes that it inhibits gastric secretion by suppressing the release of gastrin. Inhibition of acid secretion by acid bathing the pyloric gland area is also attributable to prevention of gastrin release. Both of these mechanisms for suppression of gastrin release act at the final step in the process, that is, the release of gastrin by acetylcholine. It is unlikely that hydrogen ions penetrate to the site where cholinergic release of gastrin is occurring; therefore, it is postulated that acid releases a local humoral agent which interfers with gastrin release.

Professor L. J. Witts spoke of the value of the augmented histamine test in the differentiation of the anaemias. The essential defect in pernicious anaemia was loss of intrinsic factor but in adults it was excessively rare to find free HCl in a proved case of pernicious anaemia. In only four out of 30 patients with pernicious anaemia studied did the total volume of gastric juice after histamine exceed 20 ml. Electrometric measurements revealed a pre-histamine juice more alkaline than *p*H 7·0 in all but four instances and the lowest reading was *p*H 6·71. In only two patients did the *p*H change to the acid side after histamine and the shift was less than 0·33 units. In three there was no change in *p*H. In all the others the *p*H rose after histamine and the mean maximum *p*H change indicated an increased alkalinity of 0·52 units. One of the most interesting studies at the moment was the detection of latent or preclinical forms of pernicious anaemia. As a rule

the sequence was loss of HCl, loss of intrinsic factor, and finally fall in serum vitamin B_{12}, but this order was not invariable.

Dr. Avery Jones commented on the value of the urinary pepsinogen test as a measure of gastric function.

CHEST DISEASE AND PEPTIC ULCER

B. G. RIDGEN (BRIGHTON) describes an investigation of 10,485 males of 21 years and upwards, attending a mass radiography unit, with the object of determining whether certain radiological abnormalities in the thorax were seen more commonly amongst those with a history of peptic ulceration than in those without such a history.

Each chest film was read in ignorance of the ulcer state; and each ulcer history was assessed in ignorance of the chest condition.

Of the 10,485 men, 409 (3·9%) were deemed to have a valid history of peptic ulceration. The incidence of emphysema (without radiological evidence of focal lung disease) in the ulcer group was 6·35%; in the remainder it was 1·87%. Emphysema was thus seen more than three times as frequently in the ulcer patients. Intrathoracic tuberculosis (all stages) was seen in 10·75% of the ulcer group as against 6·55% of those without an ulcer history. Congenital abnormalities (of the ribs, heart, and lungs) were seen rather less commonly in the ulcer groups than in the others.

METABOLIC INFLUENCES IN THE DISSEMINATION OF CANCER

JOHN D. GRIFFITHS (LONDON) said that the effect of dietary deficiency in producing spontaneous tumours had been well described by Tannenbaum and Silverstone, who demonstrated that prolonged restricted caloric intake and malnutrition in laboratory animals would diminish induction and growth in spontaneous tumours. However, the influence of acute metabolic changes had not been investigated in relation to increased susceptibility of the host and its possible effect on the dissemination and subsequent implantation of neoplastic cells. In the present investigation the effect of 'acute' metabolic changes, such as starvation and dehydration, which can be considered a form of metabolic 'stress', had been studied in relation to the rats' susceptibility to inoculation of Walker 256 carcinosarcoma.

Results of the experiment indicated that starvation and dehydration for 48 hours did not increase susceptibility of the rat to subcutaneous inoculation of Walker tumour, but if starvation was continued for seven days there was an increase in percentage 'takes' of the tumour as compared with the incidence in normal healthy animals. There was also an increase in 'takes' of implanted tumour in young rats.

CYTOLOGICAL STUDIES IN GASTRIC CARCINOMA

M. K. MASON (LONDON) said the investigations were being made to assess the value of cytology in the early diagnosis of carcinoma of the stomach. Two methods of approach had been employed. The first consisted of gastric lavage using normal saline in suspected or known cases of cancer in order to identify any malignant cells exfoliated from the lesions. A number of difficulties had been encountered using this technique. The second method was to make smears from all stomach immediately after resection and to examine the slides for the presence of malignant cells. By this means a number of early surface carcinomas had been identified. These growths in general cannot be recognized macroscopically, and may be very small. By making smears, a considerably wider area of mucosa is covered than is possible with sections alone. Surface carcinomas appear to be commoner than is usually realized, and are easily overlooked without careful examination of the stomach.

THE MEDIASTINUM IN PORTAL HYPERTENSION

A. E. READ AND F. DOYLE (LONDON) had carried out a radiological study of the mediastinum in portal hypertension. The 'diameter' of the azygos vein had been measured, using tomography, in a series of patients with portal hypertension and in a group of normal controls. After correction for body weight differences a significant increase in the size of this vessel had been shown to exist in the portal hypertensive group. There was, however, no apparent correlation between the size of the azygos vein and various clinical manifestations of portal hypertension, such as the presence of ascites, encephalopathy, and gastrointestinal bleeding.

Measurement of the left paravertebral shadow had also shown that in portal hypertension this is sometimes widened. This measurement is, however, probably not a reliable indication of the size of the hemiazygos veins. A very wide left paravertebral shadow is sometimes encountered in patients with ascites and may indicate the presence of fluid in the mediastinum.

Attention was drawn to other abnormal mediastinal radiological appearances, recognition of which is of importance in avoiding diagnostic errors.

CHOLECYSTECTOMY FOR CARDIAC PAIN

G. E. SOWTON considered the relationship between cardiac symptoms and gall bladder disease both theoretically and clinically, together with results of gall bladder surgery.

A preliminary report on the cardiographic changes of 40 patients undergoing cholecystectomy was produced together with the results of exercise tolerance cardiograms, blood cholesterol levels, and clinical details.

The results of experimental stimulation of the biliary tree during operation, using stronger distension stimuli than have previously been reported, were discussed, with particular reference to the cardiac risk undergone by the patient at operation.

HISTOLOGICAL ABNORMALITIES IN THE JEJUNUM AND ILEUM IN THE MALABSORPTION SYNDROME

R. HOLMES, D. HOURIHANE, AND C. C. BOOTH (LONDON) described the results of intestinal biopsies in 120 patients using the Crosby biopsy capsule. The specimens were usually examined first under the dissecting microscope and histological sections were then prepared for conventional microscopy.

Thirty patients had idiopathic steatorrhoea. In 18 there was subtotal atrophy of the villi, the biopsies appearing flat and devoid of villi under the dissecting microscope. In the other 12 patients there was partial atrophy, histological sections revealing blunt, squat, and thickened 'villi'; under the dissecting microscope these specimens showed a remarkable ridged appearance which contrasted with the baldness of subtotal atrophy. Biopsies obtained from the ileum might be normal even though there was subtotal atrophy of the jejunal mucosa.

Biopsies from four patients with chronic tropical sprue showed partial villous atrophy and a similar ridged appearance under the dissecting microscope.

The biopsy technique proved invaluable in the differential diagnosis of steatorrhoea. It was also useful in assessing the response to treatment. The procedure was safe and, apart from transient pyrexia after biopsy in two patients, there were no complications.

THE EFFECT OF HAEMATEMESIS ON THE HAEMOPOIETIC SYSTEM

W. M. DAVIDSON (LONDON) said that haematemesis was usually due to primary disease of the oesophagus, stomach, or duodenum; less frequently disease in a neighbouring organ involving the upper part of the alimentary tract; and rarely generalized disease, including diseases of the blood.

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The diagnosis and treatment of peptic oesophagitis

GEOFFREY WOOLER

From the Department of Thoracic Surgery, The General Infirmary at Leeds

SYNOPSIS An account is given of the treatment of peptic oesophagitis, in which the importance of repairing a hernia which is producing peptic ulceration is emphasized. Mobilization of the oesophagus muct be carried out as far up as is necessary for the hernia to reduce without tension.

When there is a firm stricture which will not respond to treatment, associated with shortening of the oesophagus, resection and interposing a loop of jejunum is the operation of choice which in this series has been performed without mortality.

The paper summarizes the author's experience of the treatment of peptic oesophagitis encountered amongst 1,500 patients with hiatal herniae seen in Leeds in the past 20 years. Case reports illustrate aspects of the diagnosis and medical and surgical treatment of peptic oesophagitis and stricture. The indications and limitations of dilatation of strictures either blind or under direct vision are detailed and the method of hernial repair described. Once a firm stricture has developed with gross oesophageal shortening, resection and the interposition of a loop of jejunum is the operation of choice.

During the past 20 years more than 1,500 patients have attended the Department of Thoracic Surgery in Leeds with herniae through the oesophageal hiatus of the diaphragm. The diagnosis has been confirmed in every patient radiologically and by oesophagoscopy. The examination of a patient with a hiatal hernia is not complete until oesophagoscopy has been performed, being the only way of making absolutely sure that no peptic erosion of the oesophageal mucosa is taking place. It is not uncommon to see a fairly normal looking eosophagus on the barium swallow and an intense oesophagitis at oesophagoscopy. The oesophagitis may be caused in two ways, either by the reflux of gastric contents from the stomach or by the secretion of pepsin and acid locally in the oesophagus if it is lined by gastric mucosa. Fifty patients in this series presented with a secondary anaemia, caused by bleeding from peptic oesophagitis, which had not been suspected radiologically and was only discovered by an oesophagoscopic examination. It is of extreme importance to know if there is peptic digestion of the oesophageal mucosa, so that the hernia may be repaired before the oesophageal wall becomes irretrievably damaged. The presence of oesophagitis is the main indication for advising a patient to undergo operative treatment, the other indication being the failure of medical treatment. In this series, of over 1,500 patients, all of whom were referred by other consultants for a surgical opinion, it was found necessary to operate on just over one-third of the total number after the patients had been fully investigated.

PEPTIC OESOPHAGITIS

Reduction of a hiatal hernia with restoration of the normal anatomy is not such a difficult technical procedure. However, when there has been gross destruction of the oesophageal wall by peptic ulceration there remains no real answer to the problem, other than by resection and reconstruction of the oesophagus with intestine. This often proves a long and tedious operation which cannot always be guaranteed to cure the patient's symptoms.

CLINICAL PICTURE OF OESOPHAGITIS Oesophagitis may be suspected from the history if the patient complains of heartburn, of regurgitation of acid, or of 'bile' which may produce a burning sensation behind the sternum and may rise up as high as the pharynx. Sometimes patients experience a soreness low down in the oesophagus immediately after swallowing which goes as the food passes into the stomach. Many complain of pain high in the epigastric region which is related to posture, occurring while they are lying down in bed, or when they bend down to fastern their shoes. It can often be relieved by sitting upright, and taking a hot drink or an alkaline mixture. Pain in the back is a

FIG 1a

FIG. 1b

FIG. 1c

FIG. 1d

FIG. 1. *Barium swallow obtained in a woman aged 41 years (Case 1) illustrating a hiatal hernia with a gastric-lined oesophagus (Fig. 1a). A peptic ulcer arising in this mucosa had perforated into the posterior mediastinum and produced severe back pain.*
Fig. 1b illustrates a bolus obstruction in a peptic oesophageal stricture which had produced total aphagia.
Oesophageal spasm simulating retrosternal ischaemic pain in two elderly men aged 73 (Fig. 1c) and 62 (Fig. 1d).

late symptom and denotes that the inflammatory process has spread outside the oesophageal wall and is now involving the mediastinal tissues, when sudden death may result from an ulcer perforating through the aortic wall.

Case 1 Illustrating symptoms produced by gastric-lined oesophagus A woman aged 41 years had had indigestive symptoms all her life. During the last two years she had suffered from severe substernal pain radiating into the back between the shoulder blades, which was particularly severe at night. She had also developed dysphagia and vomited all hard foods.

A barium swallow and meal showed a hiatal hernia and a long segment of oesophagus lined with gastric mucosa above which was an ulcer producing stenosis (Fig. 1a). Oesophagoscopic examination proved these findings, and biopsies from the ulcer and the mucosa just distal to it showed that it was arising in gastric mucosa.

November 1957. Operation. Extensive mobilization of the whole oesophagus in order to reduce the hernia. Bilateral vagotomy and pyloromyotomy performed. The ulcer healed. The patient is taking a normal diet and has no dysphagia. No dilatation of the stricture has been necessary.

Intermittent dysphagia is not uncommon when peptic ulceration is present, due to the lumen becoming narrower which in turn reduces the reflux. Dysphagia may also be due to hard pieces of food temporarily blocking the lumen. Bolus obstruction causes aphagia and presents with severe substernal pain and vomiting (Fig. 1b). It may sometimes be relieved with a fizzy drink such as soda water but usually requires an oesophagoscopic examination and dilatation of the stricture.

Sometimes the symptoms are first experienced when patients are lying in a recumbent position after an operation. Watkinson (1960) has had several patients develop severe heartburn while lying flat in bed after coronary infarction. Recurrent infarction was suspected, until the head of the bed was raised, and then all their symptoms disappeared, proving that their more recent pain was of oesophageal origin.

The distinction of cardiac from oesophageal pain may be difficult; the one mimics the other very closely and both may be present in the same individual, for they occur in the same age group (Fig. 1c).

Oesophageal spasm has not infrequently been treated as cardiac infarction. A barium swallow performed during an attack of pain will clinch the diagnosis of spasm in a patient who has doubtful electrocardiographic changes of infarction (Fig. 1d).

RADIOLOGICAL APPEARANCES OF OESOPHAGITIS A hiatal hernia can nearly always be demonstrated by filling the stomach with barium and then tilting the patient into the Trendelenberg position (Fig. 2a).

Reflux of barium from the stomach into the oesophagus takes place just at the end of inspiration. The peptic inflammation caused by reflux of gastric contents into the oesophagus has been aptly described by Barrett as 'reflux oesophagitis'.

While the patient is standing, barium may pass down the oesophageal lumen without demonstrating any abnormality. Later a granular appearance of the mucosa may be seen near to the cardia. This propresses into a slight narrowing and then the oesophageal wall becomes thickened. The lumen becomes narrower and a stenosis occurs which remains fixed and does not open up on swallowing more barium. An ulcer crater may sometimes be seen which denotes that it has been formed in a portion of oesophagus lined by gastric mucosa (Barrett, 1950) (Fig. 1a). Other ulcers may be present in the stomach and duodenum. Davidson (1958) has described an ulcer occurring in the hernia as it crosses the sharp edge of the hiatus of the diaphragm. He believes that it may be due to trauma caused by food passing over this edge, because it heals spontaneously after the hernia has been reduced.

Case 2 Illustrating development of a gastric ulcer in a fixed hiatal hernia A woman, aged 61 years in 1925, complained of epigastric pain and vomiting after meals. At laparotomy by Lord Moynihan, a hiatal hernia was found and repaired.

1931. Her symptoms had persisted so a second repair of the hiatal hernia was performed through the chest by another surgeon.

1944. She became much worse, having severe pain and haematemeses. A barium meal showed a large gastric ulcer at the site where the hiatal hernia crosses the diaphragm. At laparotomy there were too many adhesions to free the stomach, so the left gastric vessels were ligated. She had no further haematemeses but she was never free of indigestion and continued to have pain after evening meals, with recurrent attacks of vomiting.

1955. She was transferred to the Department of Thoracic Surgery. A barium meal showed a large hiatal hernia and a large gastric ulcer in the middle of the lesser curvature of the stomach spreading around the whole body of the stomach at the level of diaphragmatic constriction (Fig. 2b).

June 1955. Operation. Hiatial hernia repaired; sleeve resection of stomach and pyloroplasty performed. The ulcer crater measured 2 cm. in diameter and was of a simple type. She has remained well and free of symptoms since. It is possible that the ulcer might have healed after reduction of the hernia alone.

If the hernial sac is very large it may contain the whole stomach. The stomach then rotates so that its greater curve lies uppermost. Occasionally, the sac extends into the right side of the chest (Fig. 2c).

The presence of a hiatal hernia seems to make the recognition of a gastric neoplasm difficult, particularly when the neoplasm occurs in the herniated pouch of stomach.

Case 3 Illustrating development of a gastric neoplasm in a hernia A man aged 59 had had flatulence all his life, heartburn for many years, and dysphagia for the past four months.

He had attended the physicians for many years and was known to have a large hiatal hernia which had not altered radiologically. He was transferred to the Department of Thoracic Surgery when he began to lose weight and could only swallow liquids.

February 1957. He was found to have developed an adenocarcinoma in the hiatal hernia so a radical oesophago-gastrectomy was performed with jejuno-oesophageal anastomosis. He has remained well since.

OESOPHAGOSCOPIC APPEARANCES The junction of the oesophageal with the gastric mucosa will in all probability be lying above and so proximal to the pinchcock action of the diaphragm. This junction is rarely on the same horizontal plane all around the lumen, but gastric mucosa may extend up a little and so to a slightly higher level at each side.

At the onset of oesophagitis the junction is well defined even though the oesophageal mucosa is reddened. On closer examination the reddened area will reveal small vessels coursing across its surface. Later the junction of the two mucosae becomes covered with a grey exudate. If the exudate is wiped away with a gauze swab it reveals a bleeding granulating surface of a superficial ulcer. These ulcers usually encircle the oesophageal lumen and then spread up the oesophageal mucosa in a digital fashion, so that when the whole ulcerating area is viewed from above through the oesophagoscope it looks star-shaped. Distally the ulceration surrounds the whole lumen, whereas proximally it extends up the lumen in finger formation (Fig. 2d). The destructive process will have now reached the submucosa where it creates an intense fibrous reaction forming strictures. This acts as a protecting mechanism, preventing the gastric juices reaching higher up the oesophagus and destroying yet another segment. The oesophagus becomes stenosed by a thickened mass of fibrous tissue and adherent to the mediastinum with vascular adhesions.

If the ulcer has been formed in gastric mucosa lining the oesophagus it behaves like a gastric ulcer in the stomach and penetrates deeply into the mediastinum. It is this type of ulcer which may perforate into other surrounding structures. I have already mentioned one which eroded the aortic wall

FIG. 2a

FIG. 2b

FIG. 2c

FIG. 2d

FIG. 2. *Reflux of barium from stomach to hiatal hernia induced by tipping the patient into the Trendelenberg position (Fig. 2a).*
(Fig. 2b) (Case 2) In this woman, aged 61 years with a large para-oesophageal hernia, a gastric ulcer has developed at the level of the hiatus (Davidson's ulcer). Fig. 2c illustrates a large hernial sac containing the stomach, greater curvature uppermost, which is encroaching on the right lung field.
Fig. 2d is a drawing of chronic oesophageal peptic ulcer which was successfully resected in a 78-year-old man showing digitate extension of the ulcer into the oesophageal mucosa.

and more recently in another case it had perforated into the right lower lobe.

Peptic oesophagitis produces a similar lesion in children as in adults, but in children the progress is more rapid so that within six weeks of the onset of the symptoms, a stricture may have formed destroying part of the oesophageal wall. It is therefore important not to delay reduction of a histal hernia in a child if there is oesophagitis present, otherwise it may never be possible to reduce it.

ASSOCIATED LESIONS Before considering treatment it is important to find out whether there is another lesion in the stomach or duodenum causing delay in gastric emptying which in turn produces reflux oesophagitis. Many of our patients have gastric or duodenal ulcers as well as a hiatal hernia. The duodenal ulcer may be the primary lesion and when it begins to obstruct the pylorus it will cause retention of gastric contents, and reflux into the oesophagus if the hiatal mechanism is not intact. One woman presented with a giant gastric ulcer as well as a hiatal hernia.

Case 4 Illustrating association of large gastric ulcer with hiatal hernia A woman aged 74 years had had indigestion for only six years. A barium meal showed a hiatal hernia which was treated medically. She remained fairly well for three years and then developed severe epigastric pain which was periodic at first, but later became continuous and radiated into the back. The pain was so severe at times that she had to have injections of morphia.

Another barium meal showed the hiatal hernia but now there was a giant gastric ulcer which involved practically the whole of the lesser curvature and appeared to have penetrated deeply into the posterior abdominal wall (Fig. 3a).

1958. Operation. The giant ulcer involved the whole of the lesser sac but it was possible to dissect it intact from the body of the pancreas and the posterior abdominal wall. Sleeve resection of the body of the stomach was performed. The hiatal hernia was reduced and the hiatus repaired. Pyloroplasty was performed. The patient remains extremely well and has been free of all digestive symptoms (Fig. 3b).

Another patient perforated a duodenal and a gastric ulcer at the same time. Both were closed by

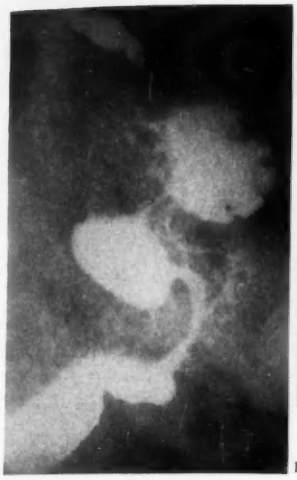

FIG. 3a

FIG. 3b

FIG. 3. *These radiographs illustrate the association of peptic ulceration with hiatal hernia. Fig. 3a shows a giant gastric ulcer which had developed below a large hiatal hernia in a 74-year-old woman (Case 4). It was found possible to remove the ulcer by a sleeve resection and reduce the hernia (Fig. 3b).*

suture and during the subsequent three weeks he developed dysphagia. A barium swallow and oesophagoscopy proved that he had an acute peptic ulcer of the oesophagus and incompetence of the cardia. He has been treated medically and all three ulcers have healed.

Two other patients were referred by general surgeons, having previously had a barium swallow and an oesophagoscopic examination. Both had easily recognizable hiatal herniae with peptic oesophagitis. However, a complete barium meal had not been performed in either case and when this was eventually carried out it revealed a pyloric neoplasm obstructing the pylorus and preventing gastric emptying.

Case 5 A woman aged 57 years had had indigestion and discomfort after food for the past five months. She also vomited occasionally. A barium swallow was performed which showed a small hiatal hernia and gastric reflux (Fig. 4a). She was oesophagoscoped and the lower oesophageal mucosa was seen to be inflamed. She was transferred to the Department of Thoracic Surgery for possible surgical repair of the hiatal hernia. A barium meal performed by Professor Johnstone showed a small pyloric neoplasm which was the cause of her symptoms (Fig. 4b).

FIG. 4a

FIG. 4b

FIG. 4. *Radiographs illustrating that peptic oesophagitis and a small hiatal hernia (Fig. 4a) can be caused by a pyloric carcinoma (Fig. 4b) which had not been recognized originally (Case 5).*

FIG. 5. *Radiographs illustrating the behaviour of the hiatus in cardiospasm. Here the heavy and dilated oesophagus may prolapse through the stretched hiatus into the abdomen (Fig. 5a) or rarely cardiospasm may be associated with a para-oesophageal hernia (Fig. 5b).*
Reflux induced by dilatation of the cardia may be set up a peptic oesophagitis as illustrated in Case 6. Here after 22 years, dysphagia the oesophagus presented the typical appearances of advanced cardiospasm (Fig. 5c).

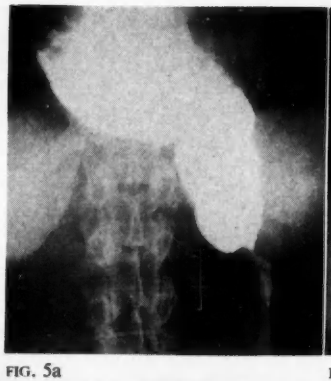

FIG. 5a

FIG. 5b

FIG. 5c

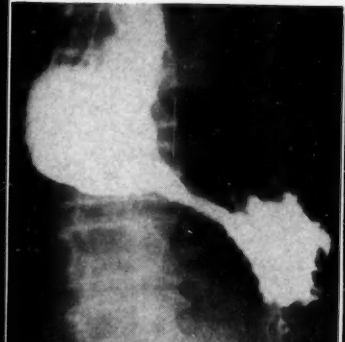

FIG. 5d

FIG. 5e

After dilatation with a hydrostatic bag a good channel was obtained through the cardia though it was rendered incompetent (Fig. 5d). One year later a severe peptic ulceration of the oesophageal mucosa had developed (Fig. 5e).

Cholelithiasis may produce the same symptoms as a hiatal hernia and occur in the same type of person. Repeated vomiting and belching caused by the presence of gallstones may so weaken the mechanism of the cardia that it eventually becomes incompetent, but otherwise there is no direct relationship between chronic cholecystitis and the development of oesophagitis.

OESOPHAGEAL HIATUS IN CARDIOSPASM During the last stage of cardiospasm when the oesophagus becomes dilated and its lumen so large that it can accommodate a full meal, the weight of this loaded oesophagus stretches the ligaments and muscles of the hiatus so that the cardia may prolapse into the abdomen (Fig. 5a). The cardiac sphincter becomes the last remaining control preventing gastric reflux, so that if this is damaged by dilatation or by an operation, peptic oesophagitis will follow. In order to prevent this from occurring, the oesophageal hiatus should be repaired at the same time as the Heller's operation, so that after the cardiac sphincter has been divided, the patient is left with a competent cardia.

Case 6 A man aged 64 years who had had 22 years of dysphagia due to advanced cardiospasm (Fig. 5c). The cardia was dilated twice with a hydrostatic bag at oesophagoscopy (Fig. 5d). He was relieved of his dysphagia for one year and then it returned in greater severity and he began to have haematemeses from which he died. He had severe peptic ulceration of the oesophagus due to incompetence of the cardia (Fig. 5e).

Rarely cardiospasm is associated with a para-oesophageal hernia and when this occurs it is obvious that the hiatus should be repaired after the hernia has been reduced and Heller's operation performed (Fig. 5b).

TREATMENT

MEDICAL TREATMENT After investigating the case fully and making sure that only minimal oesophagitis is present by oesophagoscopy, the patient may be treated medically for a time to see the response. This consists of: 1, Reduction of weight by dieting; 2, taking alkalies and antacids to reduce gastric acidity; 3, keeping to a semi-solid ulcer diet; 4, disposal of tight abdominal appliances, such as corsets; 5, the avoidance of stooping or bending positions. Sleeping well propped up with pillows to prevent reflux at night when most of the damage is done to the oesophageal mucosa (Aylwin, 1953). Patients tend to slip off pillows so it may be necessary to raise the head of the bed or become accustomed to a bed chair.

If the symptoms improve with medical treatment it may be a false impression of what is really happening unless a second oesophagoscopic examination is performed, and if this shows that the condition of the mucosa has not improved, it is a mistake to continue medical treatment any longer. In children when peptic oesophagitis is present, it is doubtful whether conservative treatment does any good at all, for it has been my experience that infants with reflux oesophagitis may produce a stricture within six weeks of the onset of their symptoms unless the cardia is made competent by operative repair.

Case 7 Illustrating rapid course of peptic oesophagitis in childhood An infant had vomited intermittently since birth. When he was 16 months old a barium swallow showed a hiatal hernia with severe peptic oesophagitis (Fig. 6a). He was treated medically and nursed in a bed chair in order to prevent gastric reflux (Fig. 6b). He appeared to make considerable improvement and seldom regurgitated food after his meals, while he was nursed in this position. A barium swallow taken after one month's medical treatment showed a dramatic improvement in the condition of the oesophagus. There was a wide lumen all the way down the oesophagus, but the cardia was completely incompetent (Fig. 6c). He was discharged home at this time his parents being told to keep him well propped up in his cot.

The improvement was not long lasting, however, for two months later, when he was 20 months old, he was admitted to the Department of Thoracic Surgery in an extremely ill condition. He had a right-sided pneumonia, most probably caused by the inhalation of food, and an empyema which had to be drained. He had developed a severe peptic stenosis of the oesophagus which required frequent dilatations in order to keep the lumen patent (Fig. 6d). At oesophagoscopy it was considered that the stricture was now fibrous and would require resecting later. The empyema healed, and the right lung cleared. He was able to take a fluid diet and was maintaining his weight, so he was allowed to go home, and it was intended to see him frequently in the Out-patient Department. However, his family moved to another town and we have not received any further information about him.

BLIND BOUGINAGE When there is an established fibrous stricture above a hernia which is not reducible, it may be considered advisable to try bouginage and treat the patient conservatively rather than perform an extensive resection. If the patient is an adult and cooperative, he may be taught to pass the bougies on himself. I have always regarded this method of treatment as rather unpleasant and not without danger; however, there is no doubt that such people can have long periods of freedom from symptoms provided they adjust their diet to pass through the stricture.

Case 8 An infant boy was slow in gaining weight and frequently regurgitated his food after meals. He appeared not to be able to swallow properly when he was 20 months old, and he sometimes vomited while he was eating.

He first attended the Infirmary when he was 2 years old, *i.e.*, in March 1948. A barium swallow at this time revealed a peptic stricture of the oesophagus and a small hiatal hernia (Fig. 7a). The stricture was short and fibrous; it required an occasional dilatation in order to keep it open, and so he continued for about a year. He then developed measles and was sent to a fever hospital. At this hospital, the dilatations were performed on him blindly using gum elastic bougies.

In June 1949 a severely emaciated and dehydrated child was transferred to the Department of Thoracic Surgery. There was complete oesophageal obstruction, for the child was unable to swallow even water. A gastrostomy was performed under local anaesthesia (Fig. 7b).

When the child had improved sufficiently to have an *x*-ray examination, a lipiodol swallow was performed which showed complete obstruction to the oesophageal lumen and a perforation leading to a mediastinal abscess, lying in the left paravertebral region. The mediastinal abscess had ruptured into the left lower lobe which was atelectatic and contained an abscess cavity. The infection had also spread into the bodies of the twelfth thoracic and first lumbar vertebrae, both of which showed evidence of osteomyelitis (Figs. 7c and d).

Gastrostomy feeding was continued for about a year. Postural drainage and antibiotic therapy controlled the infection in the lung, mediastinum, and spine. He continued to improve and rapidly put on weight. Several attempts were made at oesophagoscopy and once, retogradely, via the stomach, to try to pass a small bougie through the original lumen and stricture; all failed; the lumen at the site of the original stricture did

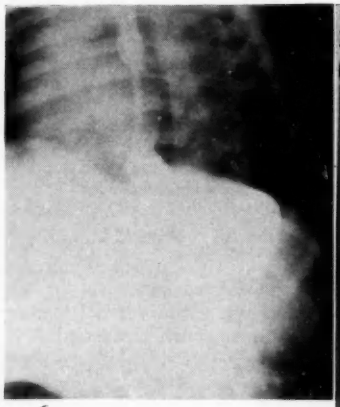

FIG. 6a

FIG. 6b

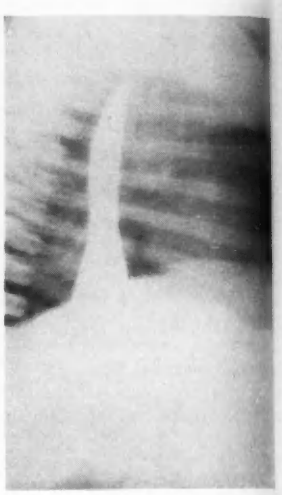

FIG. 6c

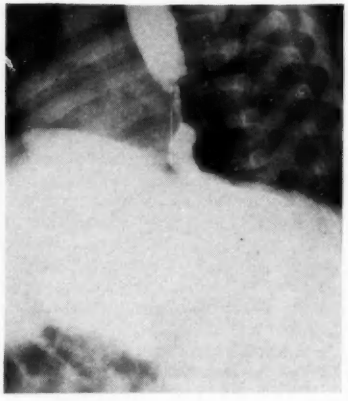

FIG. 6d

FIG. 6. *Radiographs illustrating the course of peptic stenosis in a child (Case 7) who presented at the age of 16 months with a peptic stenosis (Fig. 6a). This was treated medically by a bedchair (Fig. 6b) and appeared to effect a considerable improvement (Fig. 6c). However, four months later a firm peptic stricture has developed which required repeated dilatation (Fig. 6d).*

not appear to exist. In March 1950 a left thoracotomy was performed. There was a tremendous amount of fibrosis in the mediastinum, and the mediastinal abscess was encountered which communicated by a bronchial fistula with the left lower lobe. Bare bone could be felt with a probe at the lower extremity of the mediastinal abscess which was one of the infected vertebrae. The oesophagus was eventually dug out of the fibrous tissue in the mediastinum. The stricture was quite short and it was excised. The oesophagus was anastomosed to the small pouch of stomach forming the hiatal hernia, and this hernia was then reduced. The left lower lobe still contained an abscess with a small bronchial fistula, but it was not large and it was left alone.

Two weeks after this operation the child was able to take fluids by mouth and later solids. Three months later he required an oesophagoscopic dilatation, but this had not been necessary again. The lung abscess healed without any more trouble and also the spine.

During the past 10 years he has been taking an ordinary diet and states that he can eat anything without discomfort. A recent barium swallow was also very satisfactory (Figs. 7e and f).

Case 9 Illustrating successful outcome to self bouginage A man aged 52 years had had indigestion for about a year. In 1956 he was admitted with an acute abdominal condition and at laparotomy he was found to have perforations of both a gastric and a duodenal ulcer. The two perforations were each closed by suture. After he had been discharged home he developed dysphagia and a barium swallow showed a peptic stricture of the oesophagus (Fig. 8a). He was treated medically, but required frequent dilatations in order to keep the stricture open. He was taught self-bouginage which he continued to do for six months. After another six months on medical treatment the three ulcers appeared to heal, for he had no symptoms and the oesophagus looked normal (Fig. 8b). He has remained symptom-free for the past four years.

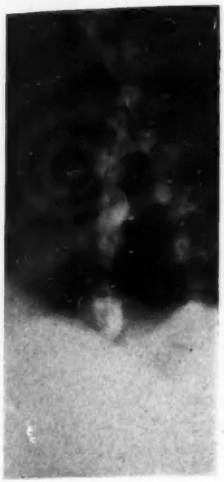

FIG. 7a

FIG. 7b

FIG. 7c

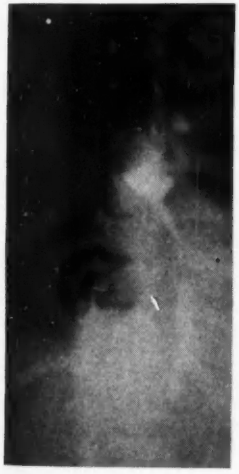

FIG. 7d

FIG. 7e

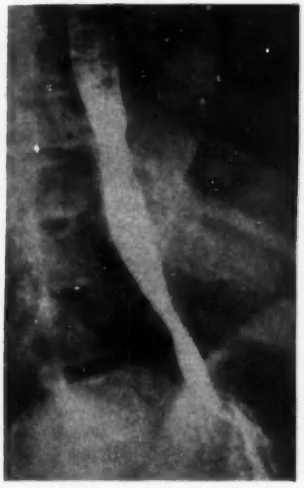

FIG. 7f

FIG. 7. *Radiographs illustrating the course of a peptic stricture which developed in March 1948 in a boy aged 2 years (Case 8). A barium swallow at that time showed the stricture with a small hiatal hernia and irregular peristalic movements (Fig. 7a). In June 1949 oesophageal perforation followed blind dilatation with the development of lung abscess, mediastinal abscess, and osteomyelitis of the spine. Fig. 7b illustrates his emaciated state at this time. A lipiodol swallow shows perforation leading into the mediastinal abscess in the left peravertebral region and the fluid level of the lung abscess is visible in the left lower lobe (Fig. 7c). The oesophagus can be seen to be completely occluded and loss of joint space between T.12 and L.1 indicates an osteomyelitis of the spine (Fig. 7d). Ten and a half years after the resection of the stricture he is quite fit, normally developed (Fig. 7e), and taking a normal diet. Barium swallow in February 1960 showed a good lumen to the oesophagus (Fig. 7f).*

8a

8b

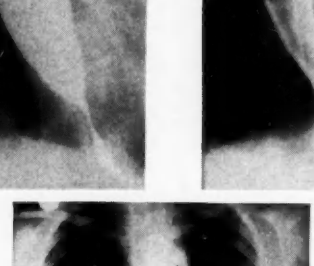

FIG. 8c

FIG. 8. *Radiographs illustrating that a successful outcome may follow dilatation of a peptic stricture by self bouginage (Case 9). After perforation of a gastric and duodenal ulcer dysphagia due to a peptic stricture developed (Fig. 8a). Repeated dilatations by self bouginage produced rapid improvement with healing of all ulcers and a return of the oesophageal lumen to normal (Fig. 8b). The oesophagus may, however, be ruptured while dilatation is being carried out under direct vision (Case 10) where the chest radiograph shows the right pleural cavity to be filled with gastric contents (Fig. 8c).*

DILATATIONS AT OESOPHAGOSCOPY This is a useful method of dealing with a stricture in a patient who is not suitable for an operation. It is less dangerous than by dilating the stricture blindly, but even when the lumen has been visualized at oesophagoscopy it has been known for the oesophageal wall to be perforated by bogies.

Case 10 Illustrating traumatic rupture of oesophagus during dilatation at oesophagoscopy (Fig. 8c) A boy aged 8 sustained a traumatic rupture of the oesophagus while a peptic stricture was being dilated at oesophagoscopy.

Two days later he was admitted to this department. At thoractomy, after the pleural cavity had been cleaned, it was difficult to find the hole in the oesophageal wall. About a pint of saline was poured into the pleural cavity and oxygen was blown down an oesophageal tube by the anaesthetist. This quickly demonstrated the leak and the hole was closed by suture. Jejunostomy was also performed.

This operation was performed eight and a half years ago. The boy has still a severe peptic stenosis and attends about every six months for an oesophagoscopic dilatation. He is awaiting admission for resection of the stricture, and reconstruction with an isolated jejunal loop.

Patients may not wish to have a big operation and prefer to have an occasional oesophagoscopic dilatation. Several elderly patients are attending this department who have continued with this method of treatment most of their lives, maintaining their weight and enjoying their meals. After a dilatation the stricture has remained open for a long time and a few have remained reasonably well for one or two years provided the peptic ulcers are not active.

Patients should be encouraged to swallow food after a dilatation, for a bolus of food is the best oesophageal dilator and is a simple way of keeping the stricture open.

Fish and hard foods should be avoided. The coarser types of fish nearly always produce obstruction as does the pulp of an orange.

SURGICAL TREATMENT

REDUCTION OF HERNIA AND REPAIR OF OESOPHAGEAL HIATUS In an article on this subject, Allison (1951) described his technique for repairing an hiatal hernia. Briefly this consists of 1, a left thoracotomy incision usually resecting the eighth rib, and a radial incision through the diaphragm to open up the peritoneal cavity; 2, division of the phreno-oesophageal ligament and hernial sac around their attachment to the edge of the hiatus; 3, mobilization of the lower oesophagus and reduction of the hernia; 4, suture of the divided sac and phreno-oesophageal ligament to the undersurface of the diaphragm; 5, making the hiatus smaller by closing the posterior part of it. This is achieved by suturing together the two muscle pillars behind the oesophagus. They in turn are formed by the right crus dividing to encircle the hiatus.

MODIFICATIONS OF ALLISON'S METHOD I have more or less followed this technique but during the last five years have modified it in the following ways.

A left thoraco-abdominal incision dividing the costal margin through the seventh or eighth costal cartilage. The rib is not resected and the pleural cavity is opened by incising the periosteum of the rib bed. This gives an excellent exposure of both the upper and the lower surfaces of the diaphragm. It carries no greater risk than a thoracotomy incision, and appears to cause less post-operative pain. It also allows one to remove a diseased gall bladder if this is necessary.

The diaphragm is opened a distance of 4 in. by dividing it near its costal attachment in a posterior direction. The incision through the diaphragm lies very near to the lateral chest wall and so avoids division of any branches of the phrenic nerve. Post-operatively it has a normal range of movement and we have not experienced difficulty in keeping the left lower lobe fully aerated.

The peritoneum is sutured and closed over the hole through which the hernia occurs, and is of prime importance when repairing any other hernia of the peritoneal cavity. This hole is the posterior part of the stretched and enlarged hiatus. Here the posterior abdominal wall is not covered with peritoneum but is a bare area lying behind the stomach and cardia, and is between the reflections of the greater and lesser sacs on to the crura of the diaphragm. It usually contains a pad of extraperitoneal fat, which slides up into the chest through the posterior part of the hiatus.

After reduction of the hernia in a similar way to that described by Allison, if one is using the thoraco-abdominal incision, it is possible to cover this bare area with peritoneum by suturing together the cut ends of the greater and lesser sac behind the oesophagus (Fig. 9). It is through this hole that the hernia recurs, so it is important to close it properly.

This repair may be further strengthened by suturing together both pleural sacs above the diaphragm. These sacs are thickened where they are in contact with the hernia and this thickening is particularly noticeable where each mediastinal pleura is reflected on to the diaphragm. These reflections hold a silk suture and make a stronger repair than suturing only the frayed muscle pillars together.

The phreno-oesophageal ligament and the hiatal sac have not a firm attachment to the oesophagus so that suturing these structures to the under surface of the diaphragm will not prevent recurrence of the hernia unless the hiatus is repaired properly at the same time.

I have operated on at least six patients in whom herniae have recurred after an Allison type of repair. In each case the ligament and sac remained sutured to the undersurface of the diaphragm but the other ends of these structures had become detached from the cardia, allowing it and a pouch of stomach to go up into the chest. Repair of the peritoneum below the diaphragm and suturing the pleural sacs together above the diaphragm appears to offer a stronger repair to the hiatus.

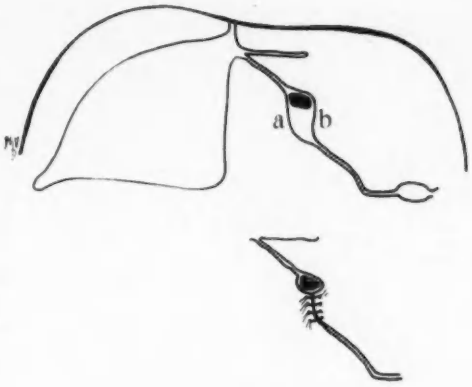

FIG. 9. *Diagram showing the peritoneal relations of the oesophagus; a is stitched to b in order to close the hole behind the oesophagus.*

This more extensive repair has proved most successful in children where previous attempts at reduction had failed (Cases 11 and 12).

Case 11 A boy weighed 10 lb. at birth. He began to vomit on the second day and continued to lose weight. When he was a month old a Ramstedt's operation was performed because he was thought to have congenital pyloric stenosis. The pylorus was normal but a large hiatal hernia was felt while the abdomen was open. He continued to vomit and lose weight. When he was 2½ months old, he weighed less than 8 lb. He was transferred to the Department of Thoracic Surgery. A barium swallow and oesophagoscopy showed a large hiatal hernia with peptic ulceration of the oesophagus (Fig. 10a).

January 1958. Operation. The hernia was repaired through a left thoraco-abdominal incision. The child immediately improved the vomiting stopped and he began to gain in weight (Fig. 10c). He has continued to improve ever since. A post-operative barium swallow shows that the hernia remains reduced and that the cardia is competent (Fig. 10b).

Case 12 A girl weighed 7¼ lb. at birth. She had vomited after almost every meal and had continued to lose weight. In July 1958 when she was 2 months old she weighed 1 lb. less than her birth weight. A barium swallow examination demonstrated a large hiatal hernia and at oesophagoscopy superficial ulceration of the oesophagus was seen (Fig. 11a).

July 1958. Operation. The hernia was reduced through a left thoraco-abdominal incision. She made excellent

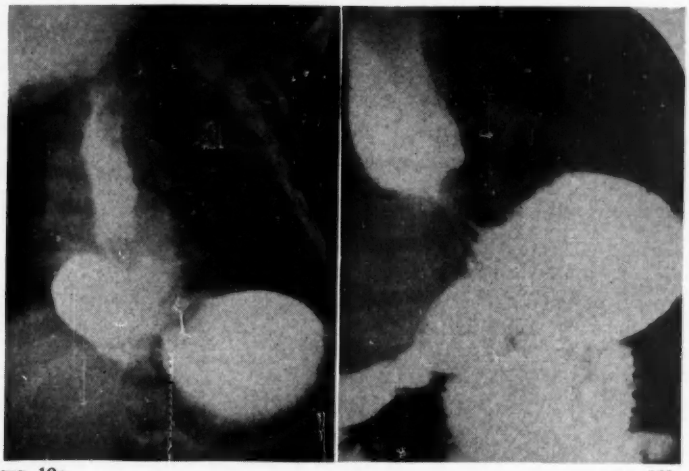

FIG. 10a FIG. 10b

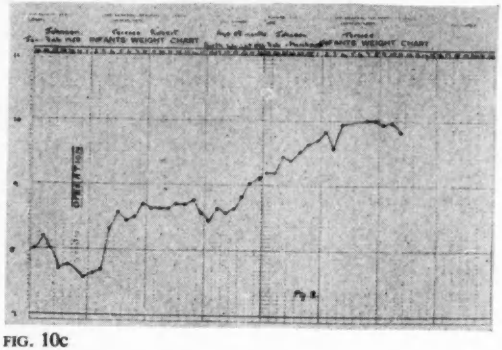

FIG. 10c

FIG. 10. *Radiographs illustrating the successful repair of a large hiatal hernia in an infant aged 2½ months (Case 11) in whom a severe peptic oesophagitis had developed (Fig. 10a). Post-operative films, eight months after the repair of the hernia, showed it to be satisfactorily reduced, though the oesophagus is slightly narrowed at the site of previous ulceration (Fig. 10b). Rapid weight gain followed the operation (Fig. 10c).*

progress. She has continued to gain in weight and has not vomited since the operation. She enjoys her food and looks very healthy. May 1960. A barium swallow examination by Professor Johnstone: 'There was no evidence of reflux and no recurrence of the hernia' (Fig. 11b).

PEPTIC STRICTURE AND SHORTENING OF THE OESOPHAGUS During the past five years I have performed few resections of the oesophagus for peptic ulceration. Instead, I have reduced the hernia by performing an extensive mobilization of the oesophagus, sometimes the whole length of it inside the chest. The advantages of leaving a patient with a physiological oesophagus rather than a poor substitute are obvious. It is well worth attempting to reduce the hernia even though the oesophagus appears shortened before embarking on a resection.

The mobilization of the oesophagus in these cases must be carried above the level of the aortic arch and even to the apex of the pleura in order that the hernia will reduce without tension. This may necessitate dividing all the branches of both vagi, which surround the oesophagus, tethering it to the mediastinum and preventing it from being mobilized adequately. If both vagi are divided, pyloromyotomy or pyloroplasty should be performed as well in order to prevent post-operative pylorospasm and interference with gastric emptying.

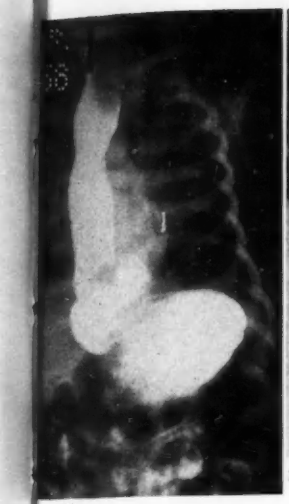

FIG. 11a

FIG. 11b

FIG. 11c

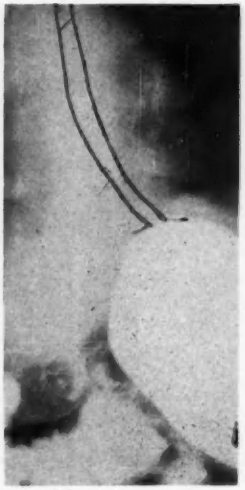

FIG. 11d

FIG. 11. *Radiographs illustrating the successful repair of hiatal hernia in children. An infant girl (Case 12) had vomited since birth and at 2½ months was 1 lb. less than her birth weight, barium swallow revealing a large hiatal hernia and peptic oesophagitis (Fig. 11a). Two years after repair a further swallow showed no evidence of reflux or of recurrence of the hernia (Fig. 11b). Successful reduction accomplished in a 10-month-old boy who had presented with hiatal hernia, peptic ulceration of the oesophagus and shortening of the oesophagus (Case 13). After repair, bilateral vagotomy, and pyloroplasty a competent cardia was obtained (Figs. 11c and d).*

The left main bronchus and the aortic arch compress the oesophagus as they cross over it, and usually prevent peptic ulceration spreading up further and to a higher level in the oesophagus. When mobilizing an oesophagus which is ulcerated, difficulty is usually encountered as high as the aortic arch. The oesophageal wall feels rigid and bound to the mediastinum with vascular adhesions. Above the aortic arch, the oesophagus feels normal and the wall will now stretch and allow the hernia to reduce. Mobilization should be continued until the hernia can be replaced below the diaphragm without tension. This may necessitate extending the thoracotomy part of the incision or making a second opening into the chest through the fourth or fifth intercostal space, in order to reach the upper part of the oesophagus. There need be no anxiety over the blood supply to the oesophagus, which is excellent, for even after full mobilization sloughing has not occurred.

This type of operation has now been performed on 18 patients all of whom had established peptic ulceration of the oesophagus and on whom a few years ago we should have considered that resection was the only surgical operation that could benefit them. The operation has proved a great success in every case, the ulcers have healed and the patients are taking a normal diet. This has not been achieved without a certain amount of anxiety in the postoperative stage, for after this operation the oesophageal lumen becomes extremely narrow where it is ulcerated. It remains narrow while the ulcer is healing, which may take six to eight weeks, and during the whole of this time the patient must be kept strictly to a fluid diet. All the patients behaved in this way: some of the ulcers healed in three weeks and then the patients were allowed a semi-solid diet, other ulcers took eight weeks, but only one patient required an oesophagoscopic dilatation. A recent follow-up examination of these patients has proved very encouraging, all of them are free of digestive symptoms and taking a normal diet.

Case 13 A boy aged 10 months had vomited since birth. He had also had two small haematemeses, the first when he was 5 days old. His mother had often seen the vomits streaked with blood.

February 1958 a barium swallow examination showed a hiatal hernia, peptic stricture of the oesophagus, and shortening of the oesophagus. He was treated medically for two months but made no progress so he was transferred to the Department of Thoracic Surgery (Fig. 11c).

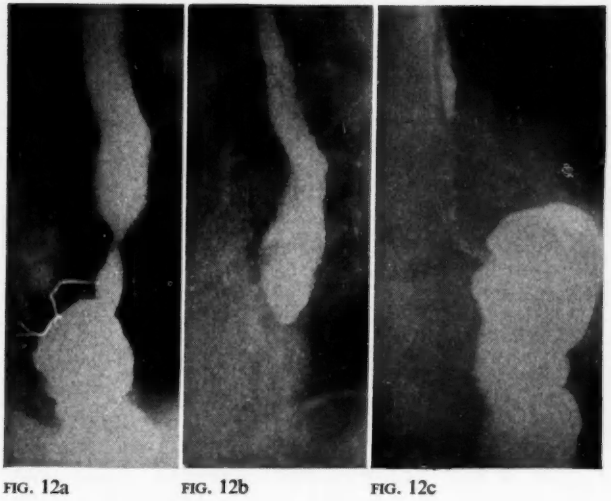

FIG. 12a FIG. 12b FIG. 12c

FIG. 12d FIG. 12e

FIG. 12. *Radiographs showing a hiatal hernia, a gastric-lined lower oesophageal segment, and peptic oesophagitis with apparent shortening of the oesophagus which developed in a woman of 48 years (Case 14) (Fig. 12a). After a year's medical treatment and dilatations the stricture had worsened (Fig. 12b) and one month after operation with reduction of hernia, pyloroplasty and bilateral vagotomy, the oesophagus is normal without reflux (Fig. 12c). In Case 15, extensive peptic oesophagitis had developed with a hiatal hernia and reached the aortic arch (Fig. 12d). Seven months after reduction, a normal oesophagus and competent cardia were present (Fig. 12e).*

April 1958. Operation. Extensive mobilization of the oesophagus in order to reduce the hernia. Bilateral vagotomy and pyloroplasty performed. The child made excellent progress after the operation. He continued to gain weight and enjoy his food.

A barium swallow taken only three weeks after the operation by Professor Johnstone: 'This appears to be an excellent result. There is no evidence of recurrence, no reflux. The oesophagus appears normal. The diaphragm moves freely. Emptying rate of stomach normal.'

The child has continued to progress well and now resents further investigations. A recent barium swallow shows that the hernia remains reduced, the oesophagus looks normal and there is no reflux (Fig. 11d).

Case 14 In 1952 a woman aged 48 years began to have dysphagia. A barium swallow performed elsewhere revealed no abnormality of the oesophagus. Her symptoms gradually became worse and in June 1954 she first attended this department. Professor Johnstone performed an *x*-ray examination and demonstrated a large hiatal hernia, peptic ulceration of the oesophagus with apparent shortening of the oesophagus, very free reflux from the stomach, and he also noted deformity of the duodenal cap.

Oesophagoscopy June 1954. Peptic ulceration surrounding the whole oesophageal lumen encountered at 30 cm. from the incisor teeth. Oesophageal mucosa inflamed as high as the aortic arch. The ulcer felt soft when bougies were passed through it, but at this time it was considered that the hernia was not reducible. She was put on medical treatment, consisting of a weight-reducing diet, alkalis after meals, etc.

August 1954. She was again oesophagoscoped and the stricture dilated because she was not able to take anything other than fluids. She lost over a stone in weight and appeared to improve for a time.

A repeat barium meal by Professor Johnstone demonstrated more deformity of the duodenal cap so that an active duodenal ulcer was considered to be present. The peptic stenosis of the oesophagus remained about the same.

February 1957. She had deteriorated during the past month and had recently had a small haematemesis. Another barium meal by Professor Johnstone: 'There is still a well marked peptic stricture above a small segment of gastric-lined oesophagus. The hiatal hernia was easily produced and reflux flooding occurs readily. The rest of the stomach is normal. The duodenal cap is much deformed and active ulceration is thought to be present. There is a moderately large diverticulum arising from the second part. The gall bladder was examined and found to be normal' (Fig. 12a).

She was oesophagoscoped and the stricture was again dilated with bougies. It still felt soft, and dilated very easily. She continued on her medical treatment and remained fairly well for a year.

In June 1958 dysphagia for fluids returned and within two weeks she was only able to take sips of water. A barium swallow showed that the oesophageal lumen was almost completely stenosed (Fig. 12b).

June 26 1959. Operation. Left thoraco-abdominal incision. The hernia was reduced after extensive mobilization of the oesophagus. All vagal fibres were divided during the mobilization. There was some scarring of the first part of the duodenum but no ulcer was seen. Pyloroplasty was performed.

After the operation she had difficulty in taking fluids by mouth during the first fortnight. Then she gradually improved so that a month after the operation a barium swallow examination showed a normal oesophagus.

She continued to improve ever since and takes a normal diet without discomfort. The last barium swallow examination performed by Professor Johnstone in March, 1960: 'The left dome of the diaphragm moved satisfactorily. The oesophagus appeared normal. No recurrence of hernia detected. No reflux' (Fig. 12c).

Case 15 In November 1950 a woman aged 50 years presented herself with several years' history of heartburn and flatulence after meals. During the past month she had had dysphagia, so that everything she took by mouth, including fluids, appeared to stick high in the oesophagus and just behind the manubrium.

23 November 1950. A barium swallow showed a hiatal hernia, with free reflux from the stomach. There was a long peptic stricture of the oesophagus reaching to above the level of the aortic arch (Fig. 12d).

Oesophagoscopy confirmed the presence of peptic ulceration which was encountered at 19 cm. from the incisor teeth. Ulceration surrounded the whole oesophageal lumen which measured only 4 mm. in diameter. The stricture felt soft when it was dilated with bougies.

She was put on medical treatment but did not improve. A further oesophagoscopic dilatation was necessary in February 1950 and again in May 1950. The appearances of the oesophagus at these examinations remained unchanged.

June 1950. Operation. The hernia was reduced after extensive mobilization of the oesophagus. The cardia was rendered competent and the peptic ulceration of the oesophagus quickly healed.

A barium swallow examination performed seven months after the operation showed a normal looking oesophagus. The hernia remained reduced and there was no reflux. She has remained well ever since, is taking a normal diet, and has no digestive symptoms (Fig. 12e).

RESECTION AND RECONSTRUCTION WITH JEJUNUM

When there is a tough fibrous stricture of the oesophagus with considerable shortening, and after other methods of treatment have failed to relieve the patient's symptoms, there is no alternative line of treatment other than to resect the diseased segment and reconstruct the oesophagus with stomach, jejunum, or colon. In Leeds, we prefer to use the jejunum for the reconstruction after an oesophagectomy, probably because we have long been accustomed to preparing jejunal loops. Since 1953 we have been using an 'isolated' or interposed jejunal loop (Allison, Wooler, and Gunning, 1957) and the operation is conducted in the following way:

A left thoracoabdominal incision is used through the bed of the eighth rib. When the operating theatre has been darkened, the jejunal mesentery is inspected with a light behind it. The first loop of jejunum near to the duodenojejunal flexure is usually selected for preparation of the isolated loop. While the light is being held behind the jejunum, the vessels in a fat-ladened mesentery can easily be seen using this method of illumination (Fig. 13a). It is rarely necessary to divide more than two of the main vessels supplying the vascular arcades, and these vessels must be divided well away from their divisions. The peritoneum and lymphatic tissue are removed from both sides of the mobilized arcades so that they hang denuded of all their coverings, almost like a garland, from the jejunal loop which may now be straightened and is no longer curved.

After the diseased portion of oesophagus, the herniated portion of stomach, and the proximal third of the stomach have been resected, the jejunal loop is brought through the mesocolon and its upper end is anastomosed to the divided oesophagus by an end-to-end anastomosis. This anastomotic line must be made as wide as possible. It may be enlarged by incising up the oesophagus anteriorly and joining the antemesenteric side of the jejunum to the top of this incision (Fig. 13b).

The loop is divided below the diaphragm. The upper end of this division which also forms the lower end of the jejunal loop is anastomosed to the remaining segment of stomach (Fig. 13c). The interposed loop between the oesophagus and stomach is now attached only by its blood supply coming from the mesenteric vessels.

The two ends of jejunum between which the loop was taken are joined together, and so the continuity of the alimentary tract is restored.

Case 16 A woman was aged 45 years when she first attended this department in 1956.

In 1912, when she was 2 years old, she drank a corrosive liquid which produced an oesophageal stricture. She had dysphagia ever since and required frequent dilatations in order to keep the lumen open.

In 1954 and 1955 she had small haematemeses. A barium swallow performed by Professor Johnstone in 1956 showed a long stricture in the middle of the oesophagus below which there was a pouch of stomach above the diaphragm, the appearance being typical of a sliding hiatal hernia with a long peptic stricture of the oesophagus (Fig. 14a). (In all probability the corrosive liquid had damaged a segment of oesophagus and prevented it from growing, so that the oesophagus became shortened, and dragged up a pouch of stomach into the thorax. I have seen peptic stenosis follow a corrosive stricture in two other patients and so I presume it is not an uncommon sequel.)

FIG. 13a

FIG. 13b

FIG. 13. *Drawings illustrating the preparation and anastomosis of the jejunal loop to the oesophageal stump. Preparation of the jejunal loop, showing division of the vessels facilitated by light shining through the mesentery (Fig. 13a). Anastomosis of jejunum to oesophagus. The oesophagus is being incised with scissors to increase the width of the anastomosis (Fig. 13b).*

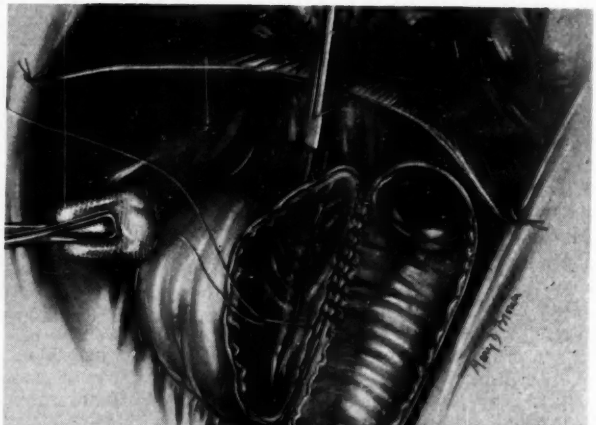

FIG. 13c

Anastomosis between the jejunum and the stomach. The jejunum has been opened longitudinally so as to make its lumen about the same size as the divided stomach (Fig. 13c).

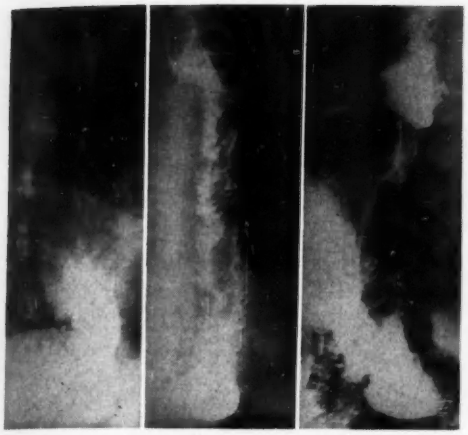

FIG. 14a FIG. 14b FIG. 14c

FIG. 14. *Radiographs illustrating the successful resection and reconstruction of a long fibrous stricture of the oesophagus produced by corrosive fluids in a 45-year-old woman (Case 16). The oesophagus is shortened and a hiatal hernia is present (Fig. 14a). After the operation of oesophago-jejuno-gastrostomy the oesophago-jejunal anastomosis can be seen with the straight loop of jejunum passing through the posterior mediastinum (Fig. 14b) leading to a wide jejuno-gastric anastomosis (Fig. 14c).*

During the next two years she was oesophagoscoped six times and the stricture was dilated each time, but she derived little or no benefit from this treatment and complained of pain in the back as well as dysphagia for all solids.

January 1959. Operation. Resection of ulcerated portion of oesophagus and reconstruction with an interposed loop of jejunum. The specimen removed contained an ulcer crater measuring 3 cm. × 2 cm. which was arising in oesophageal mucosa. The operation relieved her of the dysphagia, but she had a certain amount of abdominal discomfort after meals which was considered to be due to the division of both vagi.

A barium swallow taken four months after the operation shows: (1) The oesophago-jejunal anastomosis; (2) the almost straight lumen of the isolated jejunum as it passes down the posterior mediastinum; and (3) the wide jejuno-gastric anastomosis in the abdomen (Fig. 14b and c).

During the past year she has improved considerably and she has now little or no discomfort after meals. She has had no dysphagia since the operation and is taking an ordinary diet.

Case 17 In 1949 a woman aged 62 years attended this department. She stated that during the past seven years she had had dysphagia and she had occasionally experienced a pain in the centre of the chest which radiated into the back between the shoulder blades.

A barium swallow examination showed a stricture at the junction of the middle and lower thirds of the oesophagus associated with a hiatal hernia. She was oesophagoscoped and the appearance of severe peptic ulceration of the oesophagus was seen; superficial ulceration was evident at 20 cm. from the incisor teeth and a firm stenosis encountered at 27 cm. The stenosis was dilated with bougies. She derived no benefit from this procedure and although she was kept on strict medical treatment her symptoms did not improve.

Her general condition was only fair; she was an obese, rather frail woman who looked older than her years. Her blood pressure was 190/110 mm. Hg, she had little or no chest movement, and there was some oedema of the ankles. She was considered unsuitable for any form of operative treatment.

During the subsequent 11 years she was treated conservatively, but all this time she was gradually deteriorating and she lost over 2 st. in weight. She was never able to take anything by mouth other than fluids and she frequently had difficulty in getting these down. During this period of medical treatment she had to be oesophagoscoped 14 times and the stricture dilated.

The pain in her back became worse and continuous so that she was unable to sleep at night, and in fact her life became so unpleasant that she was prepared to take any operative risk. A barium swallow performed by Professor Johnstone had shown the peptic stenosis below which was a segment of oesophagus lined with gastric mucosa and then a pouch of stomach (Figs. 15a and b).

28 October 1958. Operation when the patient was 71 years old. Resection of the peptic stenosis, the segment of old oesophagus lined with gastric mucosa and the hiatal hernia. Oesophago-jejuno-gastrostomy performed. Pyloromyotomy was also carried out.

During the post-operative period the stomach became distended due to delay at the pylorus. The pyloromyotomy had not been complete, so the abdomen was reopened and pyloroplasty performed. She had no further trouble and made an excellent recovery.

The specimen which was removed showed a chronic peptic ulcer arising above from oesophageal mucosa and below from gastric mucosa. Below the ulcer there was a segment of oesophagus lined with gastric mucosa and then the pouch of stomach.

A post-operative barium swallow showed the oesophago-jejunal anastomosis in the mediastinum and the jejuno-gastric anastomosis in the abdomen. The pylorus now appeared widely open and there was no delay in gastric emptying (Figs. 15c and d).

She was recently seen for review as an out-patient and looked extremely well. She is taking a normal diet and has no digestive symptoms. She has gained over a stone in weight and states that she feels fitter than she did 20 years ago.

Case 18 In 1956 a man aged 66 years first attended this department with the history that five years previously he had begun to have dysphagia. This became so severe that a gastrostomy was performed at another hospital. He was able to take only clear fluids by mouth.

During the past year he had had severe pain in the back situated between the shoulder blades, which frequently kept him awake at night.

FIG. 15a

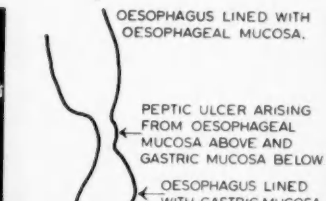
OESOPHAGUS LINED WITH OESOPHAGEAL MUCOSA.
PEPTIC ULCER ARISING FROM OESOPHAGEAL MUCOSA ABOVE AND GASTRIC MUCOSA BELOW
OESOPHAGUS LINED WITH GASTRIC MUCOSA.
HIATAL HERNIA
DIAPHRAGM
STOMACH

FIG. 15b

FIG. 15c

FIG. 15d

FIG. 15. *Barium swallow showing the presence of a peptic ulcer and stricture arising at the upper margin of a segment of oesophagus lined with gastric mucosa associated with a hiatal hernia (Case 17) (Figs. 15a and b). After resection and oesophago-jejuno-gastrostomy the oesophago-jejunal anastomosis is well shown (Fig. 15c) as is its entry to the stomach (Fig. 15d).*

FIG. 16a

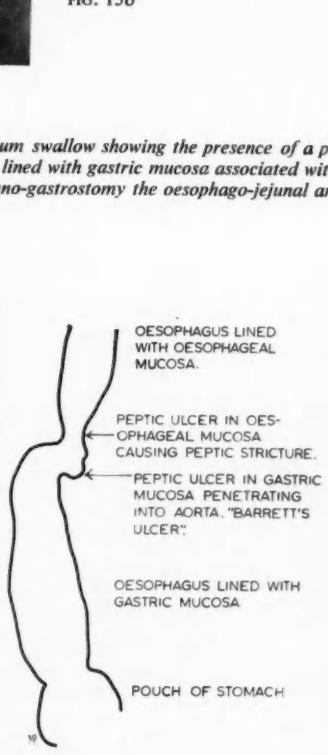
OESOPHAGUS LINED WITH OESOPHAGEAL MUCOSA.
PEPTIC ULCER IN OESOPHAGEAL MUCOSA CAUSING PEPTIC STRICTURE.
PEPTIC ULCER IN GASTRIC MUCOSA PENETRATING INTO AORTA. "BARRETT'S ULCER".
OESOPHAGUS LINED WITH GASTRIC MUCOSA
POUCH OF STOMACH

FIG. 16b

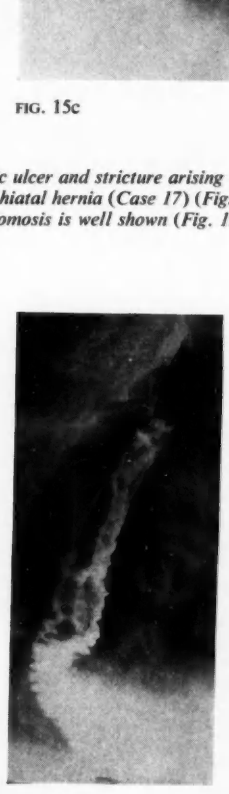

FIG. 16c

FIG. 16d

FIG. 16. *Radiographs illustrating oesophageal reconstruction in a man aged 68 years (Case 18) with peptic stenosis of the oesophagus and a Barrett's ulcer (Figs. 16a and b). Again resection and oesophago-jejuno-gastrostomy proved successful (Figs. 16c and d).*

A barium swallow performed by Professor Johnstone showed a stricture at the level of the aortic arch, below which was a segment of oesophagus approximately 15 cm. long lined with gastric mucosa. About 6 cm. above the diaphragm another expansion was evident which was obviously a pouch of stomach (Figs. 16a and 16b).

Oesophagoscopy in December 1956 confirmed the peptic ulceration at 25 cm. from the incisor teeth. The ulcer surrounded the whole lumen of the oesophagus and dilated with difficulty. Biopsies were taken of the mucosa above and below the ulcer and from the ulcer. The histology of these biopsies proved that there was gastric mucosa in the oesophagus below the ulcer, inflamed oesophageal mucosa above the ulcer, and that the ulcer was of a simple nature and not malignant.

In view of his age it was decided to treat him medically, and this form of treatment was continued for 18 months without any lasting improvement.

July 1958. Operation, when he was 68 years old. Oesophageal resection and oesophago-jejuno-gastrostomy was performed. A left thoraco-abdominal incision was used dividing the seventh costal cartilage and opening the thorax through the bed of the seventh rib. This did not give sufficient access to the site of the ulcer which was a long way up the mediastinum, so a second opening was made into the left chest through the bed of the fourth rib. The ulcer was firmly adherent to the deep side of the aortic arch and from which it proved impossible to free without mobilizing the aorta. The first four pairs of intercostal arteries were therefore divided, and the back of the aorta inspected. The base of the ulcer proved to be the far wall of the aortic arch and it was also involving the lowere end of the trachea near to the origin of the left main bronchus. It was not possible to dissect the base of the ulcer from the aorta and it was also considered unwise. The oesophagus was divided around its base, which was left adherent to the arch.

The remainder of the operation presented no difficulties and was completed in the usual way. The patient made a rapid recovery and has progressed extremely well. He has gained over a stone in weight and is taking a normal diet. He is a fairly heavy drinker and smoker; when he was seen recently, he stated that he had not had to restrict any of his activities (Figs. 16c and d).

I wish to thank Dr. Geoffrey Watkinson for his helpful criticism and advice, Professor A. S. Johnstone and Dr. M. D. Summerling for examining these patients radiologically, and Professor P. R. Allison for stimulating my interest in oesophageal surgery.

I also wish to thank Professor P. R. Allison, Mr. J. S. Davidson, and Mr. J. A. Aylwin for allowing me to review their patients, who with my own have attended this department in Leeds.

REFERENCES

Allison, P. R. (1951). Reflux oesophagitis, sliding hiatal hernia, and the anatomy of repair. *Surg. Gynec. Obstet.*, **92**, 419-431.

——, Wooler, G. H., and Gunning, A. J. (1957). Oesophagojejuno-gastrostomy. *J. thorac. Surg.*, **33**, 738-748.

Aylwin, J. A. (1953). The physiological basis of reflux oesophagitis in sliding hiatal diaphragmatic hernia. *Thorax*, **8**, 38-45.

Barrett, N. R. (1950). Chronic peptic ulcer of the oesophagus and 'oesophagitis'. *Brit. J. Surg.*, **38**, 175-182.

Davidson, J. S. (1958). Gastric ulcer in association with hiatal hernia. *Lancet*, **2**, 729-731.

Watkinson, G. (1960). Personal communication.

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The turnover and shedding of epithelial cells

Part I The turnover in the gastro-intestinal tract

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SYNOPSIS This paper confirms that the epithelial lining of the small intestine is in a state of continuous replacement and demonstrates that the whole of the gastrointestinal epithelium has a similar dynamic equilibrium.

The intestinal epithelial cells proliferate and turn over quickly. The cells divide in the basal or crypt areas and migrate up the surface of the villi from which they appear to be shed into the intestinal lumen. A number of workers have confirmed this fact in the small intestine and made observations in the stomach mucosa (Friedman, 1945; Leblond and Stevens, 1948; Stevens and Leblond, 1953). The purpose of this work is to review the epithelial turnover of the whole gastro-intestinal tract in the mouse.

The methods used for measuring the turnover of a cell population fall into two categories. Either the rate of production or destruction can be measured, since in a steady state these must be equal, or the mean cell life of the population can be determined. There is no method available for measuring the rate of destruction of the epithelial cells in the intestinal tract but the rate of production can be inferred from the number of mitoses relative to the total number of cells in the epithelium (mitotic index). Further, if the mitotic duration is known, the turnover time, *i.e.*, the time taken for replacement of the whole population of epithelial cells, can be calculated. This method has been used by Leblond and others for the small intestine.

The mean cell life can only be measured if cells can be marked with a permanent label and then followed throughout their life until they leave the population. Such labelling has been made possible by the use of radioactive substances that are specifically incorporated into deoxyribonucleic acid and therefore become a permanent nuclear label. Tritiated thymidine has been used in this method and with autoradiography it gives a visual picture of cell turnover (Leblond and Messier, 1958). The radio-labelled substance is taken up by the nuclei of cells in premitotic state (Taylor, Woods, and Hughes, 1957) and subsequently these cells, and their descendants, remain detectable by autoradiographic techniques. It is assumed in this experiment that the labelled cells behave in the same way as normal cells, although it is possible that the introduction of tritium-labelled components may result in modifications of the deoxyribonucleic acid molecule (Krause and Plaut, 1960).

Both methods indicate that the turnover of cells is extremely rapid but there is some discrepancy between the published results. The mitotic index gives a faster rate than the labelling of cells, probably due to an assumed mitotic duration which is too short. Thus Leblond and Stevens (1948), using the mitotic index, calculated a renewal rate of 1·57 days in the duodenum and 1·35 days in the ileum of the rat, whereas Leblond and Messier (1958) showed a renewal rate of three days in the small intestine of the mouse by labelling with tritiated thymidine.

The present experiments were performed on mice using labelling with tritiated thymidine.

METHODS

Eighteen-day old albino male mice were used in all experiments. Twenty-three mice were each injected intraperitoneally with 100 μc. of tritiated thymidine (A.E.R.E. Harwell: 100 mc./mM.) in 0·2 ml. saline. Animals were sacrificed at one, two, four, and six hours, and one, two, three, and five days after injection. In some the peritoneal and thoracic cavities were opened and the whole mouse fixed in Bouin's solution so that complete sagittal sections of a half mouse could be made. In the rest the gastro-intestinal tract was removed and specimens were taken from various parts of the gut and fixed in Bouin's solution. Autoradiographs were set up using stripping film (Kodak Ario), and exposed for one month. The sections were stained with neutral red after developing.

RESULTS

The labelled cells were clearly apparent with positive grains clustered over and sharply confined to the nucleus. There was no evidence of labelling of the cytoplasm.

RATE OF UPTAKE OF TRITIATED THYMIDINE Maximal uptake had occurred in one hour in all the tissues examined. No further increase in density or distribution was seen up to six hours.

DISTRIBUTION OF UPTAKE The site and number of cells labelled up to six hours indicate the cell 'production zones' and the relative amount of division occurring in them. Throughout the gut three patterns were seen.

1 In the organs lined by stratified epithelium, *i.e.*, the tongue, oesophagus, and gastric fundus, a few cells in the basal layer were labelled (Fig. 1).

2 The body of the stomach showed a specific pattern of labelling over the middle third of the glands (Fig. 2). These glands are lined by parietal

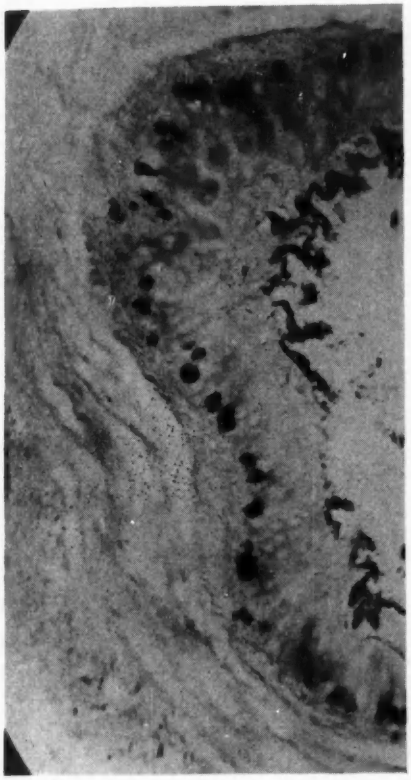

FIG. 1

FIG. 1. *Oesophagus showing labelling of nuclei in the basal layer two hours after injection 100 μc. of tritium-labelled thymidine.*

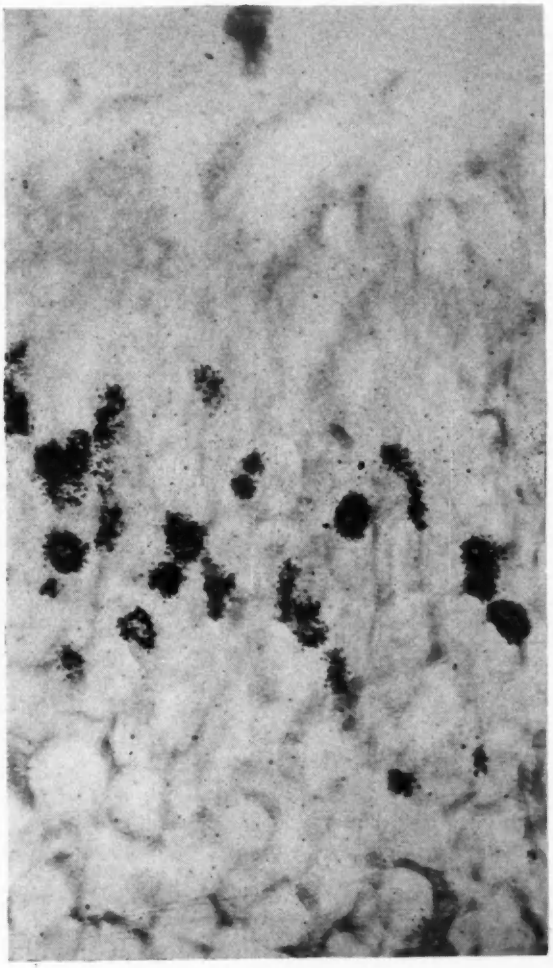

FIG. 2

FIG. 2. *Body of stomach showing labelling of nuclei in the mid-third of the glands two hours after injection.*

FIG. 3. *Jejunum showing labelling of nuclei in the crypts two hours after injection. Labelled lymphocytes are present in the villi.*

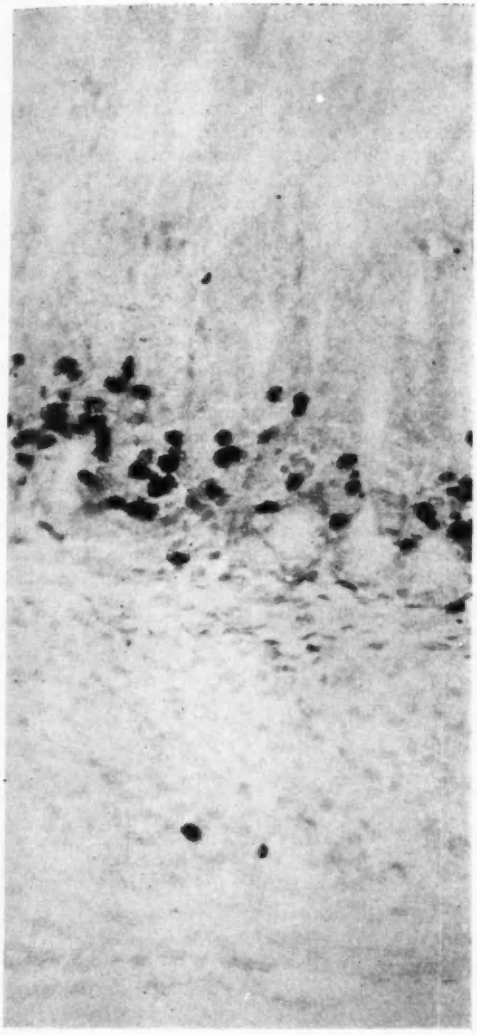

FIG. 4. *Gastric antrum showing labelling of nuclei in lower third of glands two hours after injection.*

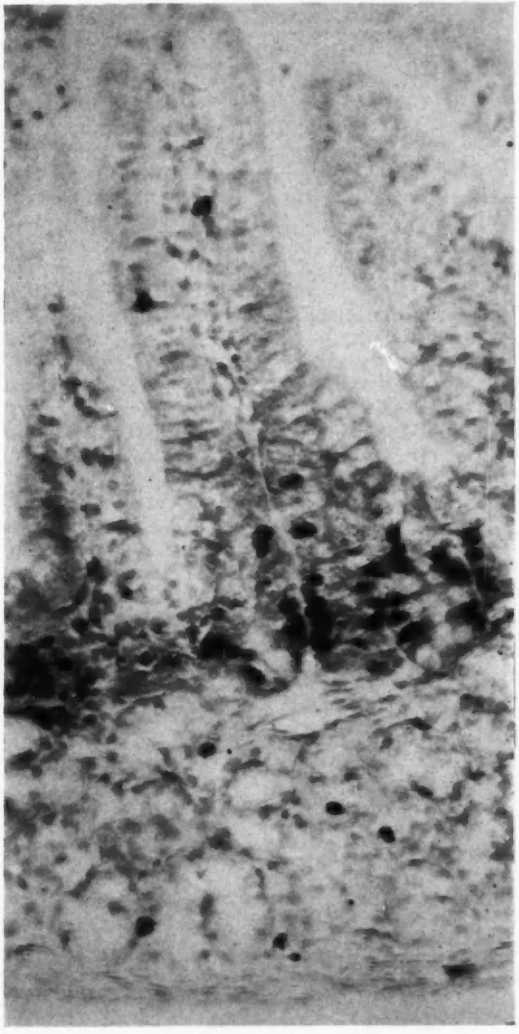

FIG. 5. *Duodenum showing labelled nuclei in crypts (above) but very scanty labelling in Brunner's glands (below) two hours after injection.*

and chief cells over the lower half and epithelial and goblet cells over the upper half but with considerable overlapping and mixing of these elements in the middle third. In the middle third a number of epithelial and goblet cells were labelled but there was no labelling of the parietal cells. In the lower third occasional cells were labelled, and these appeared to be chief rather than parietal cells.

3 The rest of the mucous epithelium of the gut showed a common pattern. In the duodenum and small intestine the cells lining the crypts were labelled (Fig. 3) and in the simple glandular epithelium of the gastric antrum and colon the cells lining the basal quarter or third of the glands were labelled (Fig. 4). In these areas most of the cells showed labelling. A few cells in the bottom of the crypts were unlabelled and these may have been Paneth or argentaffin cells but it was impossible to identify them. Brunner's glands were unlabelled except for a very occasional cell (Fig. 5).

Throughout the gut the lymphoid follicles showed the presence of labelled cells at one hour, and labelled lymphocytes were present in the mucosa in all specimens examined up to five days.

EVIDENCE OF MULTIPLICATION OF CELLS The observations at 24 and 48 hours showed an increased number of labelled cells in the gastric antrum, small intestine, and colon. For example, in the duodenum almost the entire villus was labelled at 48 hours though the crypt was nearly free of labelled cells. In contrast this increase of labelled cells was not apparent in stratified epithelium, reflecting the smaller number of mitoses.

MIGRATION OF LABELLED CELLS From the production zones the labelled cells were seen to move towards the surface of the epithelium or the tips of the villi. As has already been described a large number of cells are produced bearing the label, so it was assumed that the time taken for the first cell to reach the surface was representative of the migration time. The migration was a fairly constant phenomenon in any given tissue though there were minor variations between villi in the small intestine. The migration time of the stratified epithelia was about five days for the oesophagus and gastric fundus and somewhat over five days for the tongue (Fig. 6). In the body of the stomach labelled cells migrated from the middle third to near the surface in 24 hours, so the migration time is probably just over one day. The gastric antrum and colon showed a migration time of about one day. In the duodenum and jejunum the labelled cells travelled up the surface of the villi and the tips were reached sometime between 48 and 72 hours (Fig. 7). In the ileum, however, the tips were reached in 24 hours and it is possible that this difference is attributable to the shorter length of the villi in this part of the small intestine. Once the column of migrating cells had reached the epithelial surface or villous tip the number of labelled cells steadily diminished, although

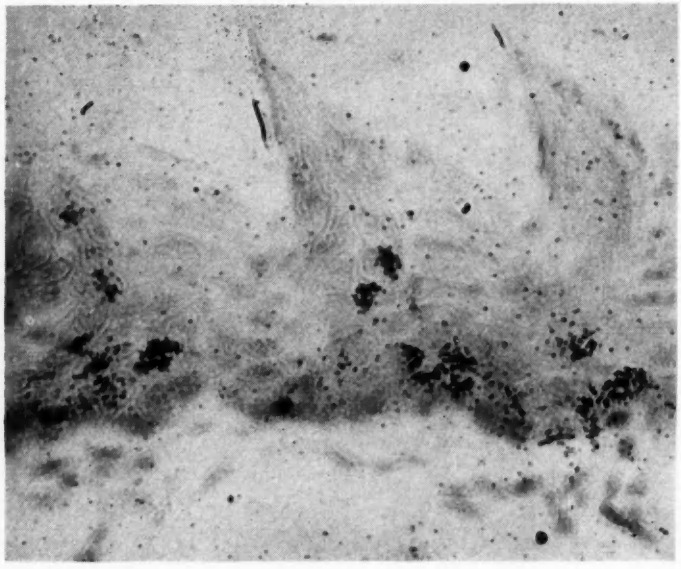

FIG. 6. *Tongue showing migration of labelled nuclei away from the basal layer two days after injection.*

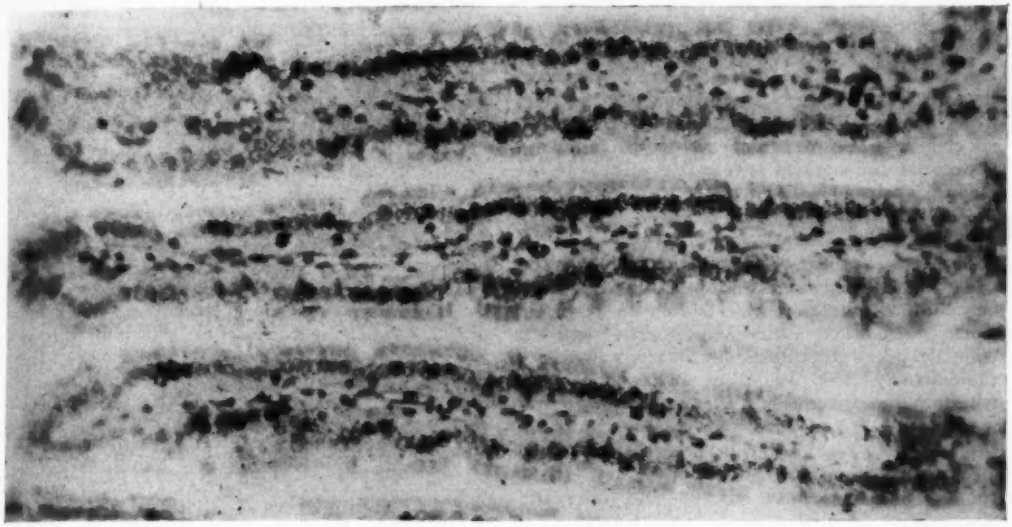

FIG. 7. *Jejunum showing labelled nuclei having migrated almost to the tips of the villi 48 hours after injection.*

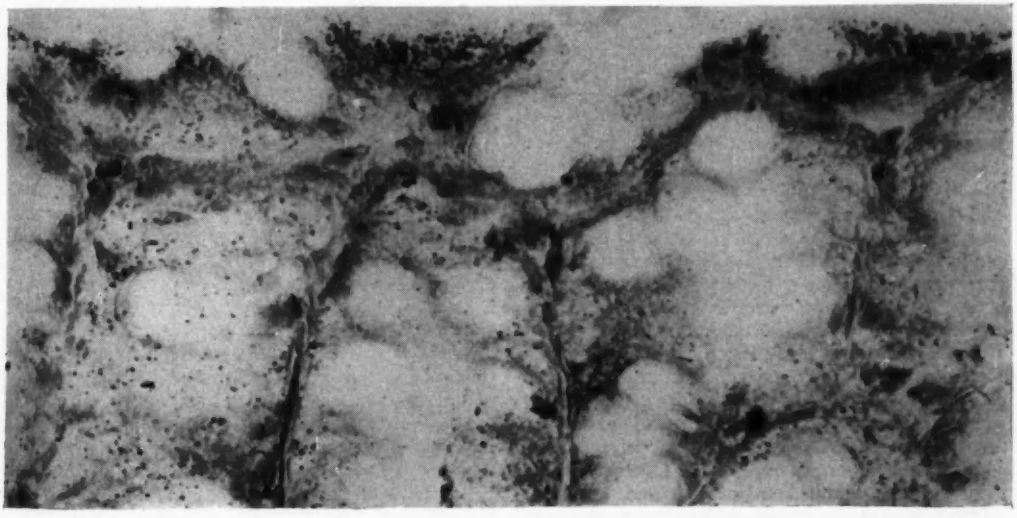

FIG. 8. *Colon showing fringe of positive grains in the cytoplasm of surface cells five days after injection.*

in the production zones a few cells remained with some labelling up to five days.

EVIDENCE OF CELL LOSS The diminution in number of labelled cells is, in itself, evidence of loss of epithelial cells. In the autoradiographs, however, it was rare to see evidence of individual cells shedding from the epithelial surface, and it was also rare to see clumps of cells in the lumen of the gut, although occasional disintegrated cells were evident with the nuclear label spilled about.

EVIDENCE OF REABSORPTION OF THE LABEL In the early stages, and during the migration of the epithelial cells, the positive grains in the autoradiographs were concentrated over the nuclei. During the period in which the labelled nuclei were disappearing, the epithelial cells were searched for the reappearance of radio-activity. This was seen only in the colon where, in the surface cells, a fringe of positive grains was present in the outer part of the cytoplasm (Fig. 8). This was evident from two to five days when all the labelled nuclei had disappeared from the colonic epithelium, but labelled cells were still disappearing from the duodenum and jejunum.

DISCUSSION

This work has confirmed the extremely rapid turnover of the gastro-intestinal epithelial cells. Although the observations were made in weanling mice the magnitude of cell proliferation was vastly in excess of that required for growth, and, in addition, the results agree well with those of other workers using adult animals (Friedman, 1945; McMinn, 1954) and the duodenal mucosa in man (Bertalanffy and Nagy, 1958).

The overall pattern that emerges is one of intense and constant proliferation in the production zones, and a steady migration of adult epithelial and goblet cells towards the lumen. The life of these cells seems to depend on the distance to be covered, the longest life being in the jejunum where the distance up the villi to the lumen is longest. In areas covered by stratified epithelium division and migration are similar but slower. The mucosa of the body of the stomach is a special case for, in this situation, the glands contain non-dividing parietal and chief cells in the lower half and actively dividing epithelial and goblet cells in the upper half. The 'production zone' is in the middle third of the glands and most of the cells appear to migrate to the surface. However, dividing and non-dividing elements are mixed in the middle third of the gland and some of these epithelial cells may be unable to migrate and may be lost into the neck region of the gland pit, as Stevens and Leblond (1953) suggest.

All workers in this field have suggested that the cells are shed into the lumen of the gut after they have reached the surface of the mucosa or the tip of the villus. This is considered in Part II. All that can be said here is that it is rare for cells to be seen in the act of shedding, and cells in the lumen are difficult to find in our preparations. This points to the swift dissolution of shed cells. It is highly probable that many of the constituents of these cells are reabsorbed, but direct evidence is lacking in the small intestine. However, in the colon the label was seen to reaccumulate in the periphery of the cytoplasm of the surface cells and was interpreted as indicating reabsorption of tritium, perhaps still incorporated with thymidine, into the epithelial cells. This appearance suggests that the colonic cells cannot handle it and that the substance piles up in the cytoplasm. In contrast, in the small intestine the tritium-labelled molecule may be rapidly absorbed so that it is not seen in the epithelial cells.

The reason for this intense division and rapid turnover of epithelial cells is not clear, but it involves the body in a vast exchange of cell mass every day. Indeed Leblond and Walker (1956) have calculated that man may lose half a pound of cells into the intestinal lumen in 24 hours. The only cells not participating in this process are the secretory cells of the stomach, Brunner's glands in the duodenum, and also the cells of the salivary glands and pancreas (Bamforth, Creamer, and Shorter, 1960).

SUMMARY

The turnover of gastro-intestinal epithelium in the mouse has been studied, using tritiated thymidine as a nuclear label and autoradiography. In the tongue, oesophagus, and fundus of the stomach the turnover time is about five days. In the body of the stomach the epithelial cells originate in the mid-third of the glands and migrate to the surface, turning over in one day. In the gastric antrum and colon the epithelial cells originate in the basal part of the glands and migrate to the surface, taking about one day for the turnover cycle. In the duodenum and small intestine the epithelial cells arise in the crypts and migrate up the villi to the tips, taking from one to about two and a half days depending on the length of the villi. Parietal and chief cells in the stomach and Brunner's glands in the duodenum do not turn over in this manner.

Part II The shedding in the small intestine

SYNOPSIS The loss of epithelial cells from the tips of villi is a visible phenomenon in the opened small intestine of the dog.

In a previous paper we have confirmed that the epithelial lining of the gastro-intestinal tract is in a state of continuous replacement. Arising by division in the crypts and basal areas of the glands the cells migrate to the surface where they disappear, it is presumed by shedding into the lumen. Leblond and Stevens (1948) have described 'streamers' of cells built up on the tips of the small intestinal villi and postulate that these are zones of extrusion where the cells are cast off into the lumen. They suggest that the villous activity assists the cells in their escape. We have been impressed by the observation that such shedding can only rarely be found in histological sections of the small intestine. Almost all the villi are covered with a continuous sheath of epithelial cells, and streamers or breaches of continuity at the tips are the exception. In autoradiographs, using tritiated thymidine as a nuclear label, shed cells were only rarely found in the lumen of the gut.

The present observations were made upon the small intestine of dogs under anaesthesia to ascertain whether shedding of cells was a visible phenomenon.

METHODS

The observations were made on 30 dogs under nembutal anaesthesia in the course of a series of experiments on villous activity. The small intestine was delivered through a median incision and opened down the antimesenteric border. The gut wall was pinned down on cork and the mucosa irrigated with Locke's solution at 38°C. The villi were observed through a Leitz dissecting microscope.

RESULTS

The villi have a regular contour (Fig. 1), though under the highest power (× 100) the outline is seen to be slightly granular. In almost every animal at some stage of the experiment an appearance was

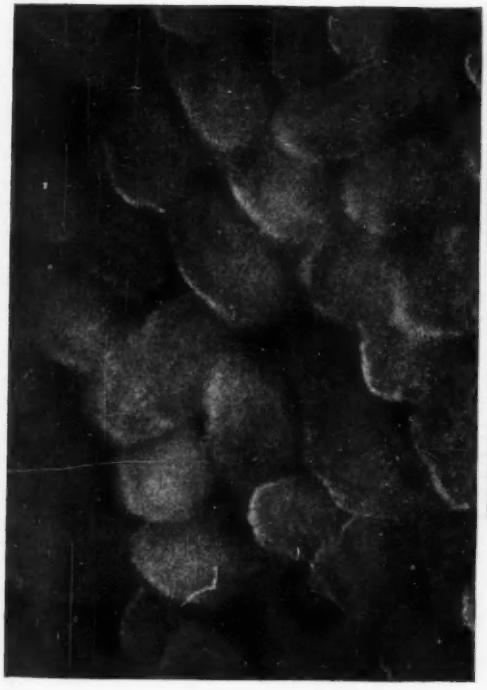

FIG. 9

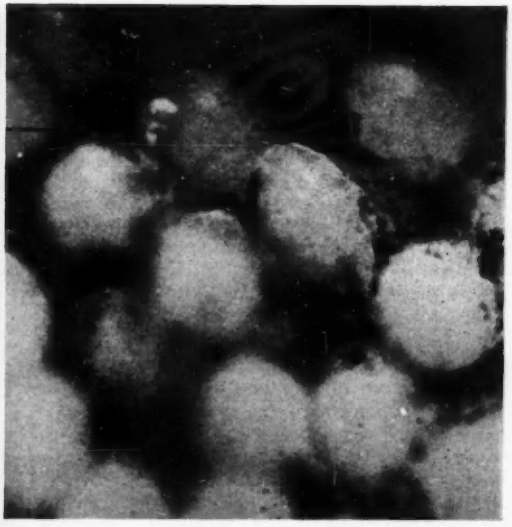

FIG. 10

FIG. 9. *Normal jejunal villi in the dog.*

FIG. 10. *Jejunal villi showing cell shedding. (A).*

seen which seemed to indicate shedding of cells. The tips of the villi appeared ragged in outline and small clear masses could be seen to detach themselves from these irregular areas (Fig. 2). These were retrieved by a micropipette and proved to be clumps of epithelial cells. This phenomenon was decidedly patchy but was observed from duodenum to ileum. There was no relation between shedding and villous activity or the length of time between the observation and opening of the gut. The appearance was present irrespective of whether the dog was fasted or fed before the experiment.

In a few animals another appearance was seen. Areas were found where most of the villous tips were capped by a white substance. Sections of these areas showed that the white caps were epithelial cells piled up into a heap several cells deep. Active shedding was not seen in these areas.

Where the mucosa had been exposed for some time or had been irrigated with Locke's solution that was warmer than usual (40°C. to 45°C.) an opaque cloud was seen to form. This was tenacious and stringy and clearly was mainly formed of mucus. The opaque mucus was aspirated and fixed on a slide and stained. It contained vast numbers of 'sheaths' of epithelial cells which were complete epithelial casts of villi. These must have desquamated from almost the whole length of the villi. They contained no substance of the core of the villi.

DISCUSSION

The shedding of small intestinal cells into the lumen of the dog's intestine is clearly visible but the mechanism is not clear. It is not related to villous activity and, indeed, the turnover of cells in the stomach and colon, where there is no activity comparable to the villous contractions, is as fast as in the small intestine. Shedding also appears to be an intermittent phenomenon.

The reason for the prodigious turnover of cells is difficult to understand. The replacement for loss from wear and tear is an obvious explanation but the appearance of spontaneous shedding suggests that the proliferation is in excess of this need. A second explanation could be that cell shedding is, in some way, related to the function of secretion of mucus and mucus may be actively secreted by the process of cells shedding. In support of this are the observations of marked mucus secretion associated with massive cellular desquamation, although of course, the experimental conditions under which it was seen make one hesitate before assuming that it happens in the intact animal.

SUMMARY

In the dog it is possible to see the shedding of clumps of epithelial cells from the tips of the villi. Occasionally caps of heaped up epithelial cells are visible on the tips of the villi.

Abnormal conditions provoke an excessive outpouring of mucus and this is associated with massive desquamation of the epithelial covering of the villi.

We wish to thank Professor R. C. Curran for much help and encouragement in these experiments and Mr. A. E. Clark and Mr. R. G. Lack for technical assistance.

REFERENCES

Bamforth, John, Creamer, B., and Shorter, R. G. (1960). Unpublished observations.

Bertalanffy, F. D., and Nagy, K. (1958). The mitotic activity and renewal rate of human duodenal epithelium. *Anat. Rec.*, **130**, 271-272.

Friedman, N. B. (1945). Cellular dynamics in the intestinal mucosa: the effect of irradiation on epithelial maturation and migration. *J. exp. Med.*, **81**, 553-557.

Krause, M., and Plaut, W. (1960). An effect of tritiated thymidine on the incorporation of thymidine into chromosomal deoxyribonucleic acid. *Nature* (*Lond.*), **188**, 511-512.

Leblond, C. P., and Messier, B. (1958). Renewal of chief cells and goblet cells in the small intestine as shown by radioautography after injection of thymidine-H^3 into mice. *Anat. Rec.* **132**, 247-257.

——, and Stevens, C. E. (1948). The constant renewal of the intestinal epithelium in the albino rat. *Ibid.* **100**, 357-377.

——, and Walker, B. E. (1956). Renewal of cell populations. *Physiol. Rev.*, **36**, 255-276.

McMinn, R. M. H. (1954). The rate of renewal of intestinal epithelium in the cat. *J. Anat.* (*Lond.*), **88**, 527-532.

Stevens, C. E., and Leblond, C. P. (1953). Renewal of the mucous cells in the gastric mucosa of the rat. *Anat. Rec.*, **115**, 231-243.

Taylor, J. H., Woods, P. S., and Hughes, W. L. (1957). The organization and duplication of chromosomes as revealed by autoradiographic studies using tritium-labeled thymidine. *Proc. nat. Acad. Sci.* (*Wash.*), **43**, 122-128.

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The influence of cortisone on the parietal cell population of the stomach in the dog

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SYNOPSIS Two experiments were undertaken in dogs to discover the influence of cortisone on the parietal cell population of the stomach.

In one, samples of mucosa were removed at operation before and after the administration of cortisone in an average total dose of 39 mg. per kg. (in seven days). The thickness of the mucosa was increased by 16% and the number of parietal cells per unit area was increased by 33%.

In the other experiment different doses were given by daily intramuscular injection for various periods. The dogs were killed and their whole stomachs were examined. Cortisone in an average total dose of 28 mg. per kg. (in three to seven days) increased the mean mucosal thickness by 27%, increased the number of parietal cells underlying a unit area of the gastric mucosa by 50%, and the total parietal cell population by a similar proportion. It did not influence the area of the gastric mucosa. Smaller doses of cortisone were less effective and larger ones tended to reduce the mucosal thickness and the number of parietal cells.

We have reported previously that pharmacological doses of cortisone increase by about 50% the maximal acid secretory response to histamine of denervated gastric pouches in dogs (Clarke, Neill, and Welbourn, 1960). Similar observations have been made with the augmented histamine test in patients receiving cortisone, prednisoline, and ACTH (Crean, 1960). A possible explanation of this finding was provided by our preliminary observation that cortisone and methyl prednisolone appeared to increase by about 50% the number of parietal cells in the gastric mucosa. The two experiments reported here were designed to provide more information about the effects of cortisone on the parietal cell population of the stomach.

MATERIALS AND METHODS

In the first experiment six mongrel dogs, weighing 8 to 10 kg., were used. A biopsy of the whole thickness of the stomach wall, measuring about 2 cm. × 1 cm., was taken from each at open operaton. The biopsy was taken from the centre of the anterior wall in three and from the centre of the posterior wall in the other three. Each dog was then injected intramuscularly with cortisone acetate, 50 mg. daily for seven days, at the end of which time a second similar biopsy was taken from the opposite wall of the stomach. The dose of cortisone was a little higher than that of the moderate range in the second experiment (Table I).

The biopsies were flattened with the fingers until the mucosa was smooth, pinned out on cork, and fixed in dilute corrosive formol for one day. Vertical sections were then cut (at 7μ thickness), stained with haematoxylin and eosin and examined microscopically (4 mm. objective) with the help of an eyepiece micrometer. The total mucosal height (surface to muscularis mucosae) was measured at 10 representative sites and the number of parietal cell nuclei contained between two lines at right angles to the mucosal surface and 0·25 mm. apart was counted. The average mucosal height and the average number of parietal cells underlying a unit area (7 μ × 1,000 μ) of mucosa were calculated for each specimen. The 'crude' parietal cell counts obtained by this method are higher than the true counts because they include parts of nuclei which extend into adjacent microtome sections. The 'corrected' counts were estimated by the method of Abercrombie (1946).[1]

In the second experiment 28 mongrel dogs, weighing between 8 and 15 kg., were used. Nine served as controls and 19 received cortisone acetate by daily intramuscular injections of either 100 mg. or 50 mg. for different periods. The doses (in terms of mg. per kg. of body weight) and the times for which the cortisone was given are shown in Table I.

At the end of the experiment the dogs were killed by electrocution. The stomachs were removed immediately

[1]The calculated correction factor for nuclear overestimation (Abercrombie's formula 1) was 0·51. The mean observed diameter of the parietal cell nuclei ((largest + smallest) ÷ 2) was 6·68μ. It was the same in vertical and horizontal sections and was not influenced by cortisone.

TABLE I

DETAILS OF CORTISONE ADMINISTRATION

Group	No. of Dogs	Average Daily Dose (mg. cortisone per kg. body weight)	Time between First Injection and Removal of Stomach	Average Total Dose of Cortisone (mg./kg.)
Experiment I				
—	6	5·6	7 days	39
Experiment II				
I (Control)	9	—	—	—
II ('Low' dose)	2	7·7	6 hours	11
	2	8·3	24 hours	
	2	8·7	48 hours	
III ('Moderate' dose)	4	8·9	3 days	28
	2	4·3	7 days	
IV ('High' dose)	2	8·7	7 days	90
	5	10·2	10 days	

and treated in a manner similar to that described by Cox and Barnes (1945) and by Cox (1952). They were cut open along the greater curvature and adherent food was washed off with physiological saline. They were then stretched and pinned out on cork boards, so that as many as possible of the mucosal folds were flattened, and finally fixed in dilute corrosive formol for 24 hours.

After fixation the outline of the stomach was traced on paper. Three strips, about 1 cm. wide, were cut from the entire length of each stomach, one from the lesser curvature (between the oesophagus and the pylorus) one from the middle of the anterior wall, and one from the middle of the posterior wall. The mucosa (together with the muscularis mucosae) of each strip was separated from the muscular coat and rolled up longitudinally, with its superficial surface inwards, embedded in paraffin wax, sectioned (at 7 μ thickness), and stained with haematoxylin and eosin. In this way three longitudinal sections of each stomach, 7μ thick, were obtained.

The junction between the mucosa of the pyloric antrum and that of the body of the stomach was seen clearly under the microscope in each section and its distance from the pyloric end of the strip was measured. The positions of these junctions were marked on each tracing and straight lines were drawn from the point on the lesser curvature, through those on the dorsal and ventral walls, to the edges of the tracing. The area of the acid-secreting portion of each stomach (body plus fundus), thus delineated, was measured with a planimeter.

Ten representative sites from each section were examined microscopically (16 mm. objective) without knowledge of whether or not the dog had received cortisone. At each site the total mucosal height was measured and the number of parietal cell nuclei between two lines 0·34 mm. apart was counted. From these figures the average total mucosal height and the average corrected parietal cell count per unit area (7 μ × 1,000 μ) of mucosal surface were calculated for each section as in the first experiment. Average values for each stomach were estimated from each set of three sections. Shrinkage of the sections after fixation (*i.e.*, during the process of separation of the mucosa, rolling, embedding in wax, and staining) was found, by measurement, to be negligible and was ignored.

Three estimates were therefore available for each stomach: (1) The average mucosal height; (2) the average corrected number of parietal cells underlying an area of mucosal surface measuring 7 μ × 1,000 μ; and (3) the area of the acid-secreting mucosa. From these figures (2 and 3) the total number of parietal cells in the stomach was established.

RESULTS

EXPERIMENT I (Table II) Cortisone increased the height of the mucosa in each of the six stomachs, the mean increment being 16%. The number of

TABLE II

INFLUENCE OF CORTISONE ON TOTAL HEIGHT OF GASTRIC MUCOSA AND ON NUMBER OF PARIETAL CELLS IN STOMACH (EXPERIMENT I)

Dog	Total Height of Mucosa (μ)			No. of Parietal Cells per 7,000 sq. μ. of Mucosal Surface		
	Before	After	Difference (After-Before)	Before	After	Difference (After-Before)
1	600	620	+20 (3%)	91·0	97·5	+ 6·5 (7%)
2	645	695	+50 (8%)	57·5	79·1	+21·6 (38%)
3	590	680	+90 (15%)	61·2	84·0	+22·8 (57%)
4	440	630	+190 (43%)	25·7	48·3	+22·6 (88%)
5	530	670	+140 (26%)	45·3	60·2	+14·9 (33%)
6	715	740	+25 (3%)	107·0	100·0	− 7·0 (7%)
Mean	587	673	+86[1] (16%)	64·6	78·2	+13·6[2] (33%)

[1] t (paired data) = 3·10 D of F = 5 0·05>P>0·02
[2] t (paired data) = 2·78 D of F = 5 0·05>P>0·02

TABLE III

INFLUENCE OF CORTISONE ON TOTAL HEIGHT OF GASTRIC MUCOSA AND ON NUMBER OF PARIETAL CELLS IN STOMACH (EXPERIMENT II)

Group	*No. of Dogs*	*Mean Total Height of Mucosa (μ)*	*Mean No. of Parietal Cells per 7,000 sq.μ of Mucosal Surface*	*Mean Area of Acid-secreting Mucosa (sq. cm.)*	*Mean Total No. of Parietal Cells in Stomach (millions)*	
					Per Dog	*Per kg. of Body Weight*
I (Control)	9	590±20 (a, b)	66·9±6·5 (c)	278±22	273±36 (e)	24·2±2·2 (f)
II ('Low' dose)	6	707±49 (a)	77·4±2·8 (d)	316±31	348±55	27·5±1·3
III ('Moderate' dose)	6	750±25 (b)	100·1±5·1 (c, d)	248±41 (284±53)[1]	358±65 (409±43)[1] (e)	31·5±5·9 (36·1±4·1)[1] (f)
IV ('High' dose)	7	613±63	62·7±10·3	286±34	275±71	25·4±5·2

Figures following ± signs are standard errors of the means.
[1]Excluding one stomach of very small area (see text).
Statistical Analyses:

	t	D.F.	P		t	D.F.	P		t	D.F.	P
a	2·51	13	0·05>P>0·02	c	3·77	13	0·01>P>0·001	e	2·27	12	0·05>P>0·02
b	5·00	13	P<0·001	d	3.92	10	0·01>P>0·001	f	2·70	12	0·02>P>0·01

Other differences between means are not significant at the 5% level.

parietal cells per unit area was increased in five of the six stomachs, the mean difference being 33%.

EXPERIMENT II (Table III) The results have been analysed in terms of three main ranges of total dosage, which we have called 'low', 'moderate', and 'high' (Table I). The differences between the sub-groups are small and insignificant in comparison with those between these three main groups.

The total height of the mucosa varied very little in the control animals and its mean was similar to that of the control specimens in the first experiment. It showed a mean increase of 20% with the low dosage of cortisone (group II) and of 27% with the moderate dosage (group III). The high dosage (group IV) did not affect the mean height, although it produced a very large range of measurements.

An increase in the mean number of parietal cells underlying a unit area (1,000 μ × 7μ) of the mucosal surface accompanied (or followed) the thickening of the mucosa (Fig. 1). The effect was insignificant with the low dosage (group II), but there was an increment of 50% with the moderate dosage (group III). The high dose again produced a large range of counts, but did not influence the mean (group IV). Of the seven counts one was above the control range, suggesting stimulation, and two were below the control range, indicating inhibition.

The area of the gastric mucosa varied greatly in all groups. The variation was not related to that of the height of the mucosa or of the numbers of parietal cells, and the mean area was not influenced significantly by cortisone. The mucosa of one dog in group III had an area far smaller than that of any other dog, although it was normal in other respects. (It measured 67 sq. cm., the next smallest, one of the controls, being 165 sq. cm.) If this dog is excluded from the analysis (as seems reasonable) the total number of parietal cells in the stomach (the product of the area × the number of cells per unit area) showed mean changes very similar to those of the number of cells underlying a unit area, the increment again being 50% with moderate doses of cortisone (group III). If the dog is included the increase in the mean is insignificant. Calculation of the number of parietal cells per kilogram of body weight makes little difference to the results.

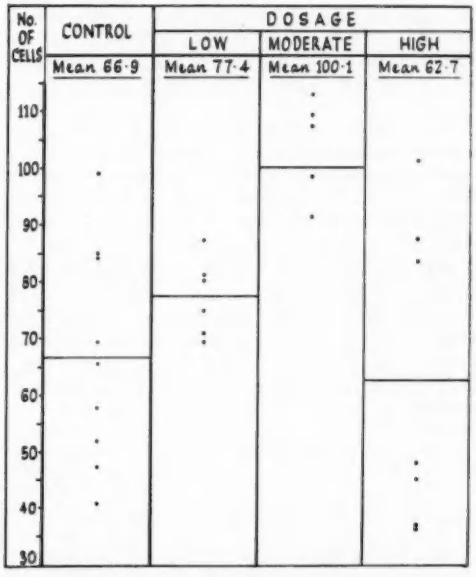

FIG. 1. *The influence of cortisone on the number of parietal cells per 7,000 sq. μ of gastric mucosal surface.*

DISCUSSION

There can be little doubt that cortisone, within the range of dosage which we have called 'moderate', causes an increase of about 50% in the number of parietal cells in the stomach. The time of administration is, however, a critical factor. Thus a daily dose of 8·7 to 8·9 mg. per kg. for two days had little, if any, effect while in three days it caused a definite increase; in seven days the effect was no longer apparent. It seems probable that the high total doses (8·7 mg. or more per kg. per day for seven to 10 days) caused an increase initially and then a reduction. All the animals in the high dose group (IV) had passed through a phase in which the total dosage had been 'moderate' and presumably they had had a uniform increase in their parietal cells at this time. By the end of the experiment some had very high cell counts and others had very low ones. The increases in the cell count and in the mucosal heights were rather smaller in the first experiment than in the second and showed considerable variation. The explanation may be that the sampling of the gastric mucosa was much less extensive.

It is likely that the increase in the parietal cell population represents a 'work hyperplasia', for similar increases have been observed in the guinea-pig (Cox and Barnes, 1945) and in the dog (Tongen, 1950) after prolonged stimulation with histamine. The significance of the increase in mucosal height is not clear. The increase in the number of parietal cells may be partly responsible for it, but detailed information about the other cellular elements and the interstitial tissues is not available.

The average number of parietal cells per unit area in the control dogs in this investigation was about one-third of the number found by Tongen (1950) and by Marks, Komarov, and Shay (1958) in dogs of similar weight, and the average total number of parietal cells in the stomach was about one fifth of the number found by the latter authors.[1] The numbers of cells in our dogs were similar to those found in patients with gastric ulcer by Card and Marks (1960), while the numbers in the other investigations were similar to those in patients with duodenal ulcer. There is no obvious reason for this difference. The cell counts in our two experiments were made independently (that in the first by R.M.H. and that in the second by N.C.R.W.R.) and the means of the two control groups agreed closely (64·4 and 66·9 respectively, Tables II and III). If there was a systematic error in our observations it is unlikely to have influenced the relative estimates, although it would have affected the absolute ones.

It is interesting to note that there is a general similarity between these effects of cortisone on the parietal cell population and those which we noted previously on the secretion of acid. This is seen in the level of dosage which causes stimulation, in the latent period of about three days before a definite effect is observed, and in the inhibitory action of large doses. Finally, the increase in parietal cell population is very similar to that of the maximal acid secretory response to histamine. It is reasonable, therefore, to assume a cause-and-effect relationship between them.

[1]To avoid confusion it should be pointed out that Marks *et al.* used 'billion' in the French or American sense of '10^9' and not in the British sense of '10^{12}.'

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REFERENCES

Abercrombie, M. (1946). Estimation of nuclear population from microtome sections. *Anat. Rec.*, **94**, 239-247.

Card, W. I., and Marks, I. N. (1960). The relationship between the acid output of the stomach following "maximal" histamine stimulation and the parietal cell mass. *Clin. Sci.*, **19**, 147-163.

Clarke, S. D., Neill, D. W., and Welbourn, R. B. (1960). The effects of corticotrophin and corticoids on secretion from denervated gastric pouches in dogs. *Gut*, **1**, 36-43.

Cox, A. J. (1952). Stomach size and its relation to chronic peptic ulcer. *A.M.A. Arch. Path.*, **54**, 407-422.

——, and Barnes, V. R. (1945). Experimental hyperplasia of the stomach mucosa. *Proc. Soc. exp. Biol. (N.Y.)*, **60**, 118-120.

Crean, G. P. (1960). The effects of A.C.T.H. and corticosteroids on gastric secretion in humans. *Gut*, **1**, 82.

Marks, I. N., Komarov, S. A., and Shay, H. (1958) Acid secretory responses to histamine and the parietal cell mass in the dog. *Amer. J. Physiol.*, **195**, 528-534.

Tongen, L. A. (1950). The quantitative relationship between parietal cells and gastric acidity. *Surgery*, **28**, 1009-1015.

Gut, 1961, **2**, 123

The measurement of gastrointestinal protein loss by a new method

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SYNOPSIS This paper reports a new method of estimating protein loss from the alimentary tract using radio-iodinated human serum albumin (RIHSA). This has advantages over the use of ^{131}I-PVP.

The term 'exudative enteropathy' (Gordon, 1959) has been applied to conditions in which protein may be lost from the body by passing through the intestinal wall. Several methods have been employed to study this phenomenon. Paper electrophoresis and immunophoresis have been used to determine the presence of autogenous human albumins and globulins in intestinal juices (Schwartz and Jarnum, 1959; Gross, Embree, Bally, Shipp, and Thorn, 1960). Such methods are mainly qualitative. Gordon (1959) used radio-iodinated polyvinyl pyrrolidone, and Schwartz and Thomsen (1957) used radio-iodinated human serum albumin (RIHSA) in attempts to study intestinal protein loss quantitatively. Polyvinyl pyrrolidone (PVP) is a synthetic polymer of molecular weight averaging about 40,000. It has been used as a plasma substitute but unlike albumin it is unaffected by digestive juices. It is thought that it cannot be absorbed from the alimentary tract. These properties make it possible to recover in the faeces PVP excreted into the gut.

In a study of patients with idiopathic hypoproteinaemia Gordon (1959) showed that after an intravenous injection of ^{131}I-PVP the faeces contained an abnormally large amount of it. Similar results were obtained by Schwartz and Jarnum (1959) in a case of hypertrophic gastritis. These results indicated that hypoproteinaemia in these cases was associated with an increased excretion of a macromolecular substance similar physically to plasma albumin (molecular weight 66,000), and suggested increased excretion of protein into the gastrointestinal tract.

There are difficulties in the use of PVP. It is possible that it may sometimes be partially reabsorbed. It is selectively trapped by the reticuloendothelial system. It does not have a uniform molecular size, and it may be that different sized molecules are excreted at different rates in the gut, as in the kidney. There are facets of protein excretion which the use of PVP cannot illuminate. For example, the magnitude of PVP excretion does not necessarily equal that of protein excretion as the two are not chemically comparable. Furthermore, PVP excretion may not be increased in states of increased protein loss due to excessive mucosal desquamation, or to alteration in the quantity, or protein content, of intestinal secretions.

This paper describes a new method of studying protein loss in the gut using RIHSA, which does not have the disadvantages of ^{131}I-PVP. With this technique patients with hypoproteinaemia and oedema have been found with increased RIHSA excretion but normal ^{131}I-PVP excretion.

MATERIALS AND METHODS

Details of the 17 patients studied are given in Table III where they are shown divided into four groups.

PROTEIN TURNOVER This was studied by the use of RIHSA. The material was obtained from the Radiochemical Centre at Amersham. It was manufactured by a modification of the jet method of McFarlane (1956). The specific activity of the material was about 63 μc. per mg. The degree of iodination was one atom of iodine to each two molecules of albumin. The material was diluted and mixed with albumin to give a final concentration of 2 mg. of albumin per ml. containing 2 μc. of activity. The method of studying protein turnover was that of Berson, Yalow, Schreiber, and Post (1953).

1 The patient is given Lugol's iodine 10 minims b.d. for two days before the test and for its duration.

2 From 20 to 30 μc. of RIHSA is injected intravenously.

3 Blood is taken in a heparinized syringe 10 minutes after the injection to obtain the initial activity after plasma distribution has occurred.

The plasma radioactivity is determined daily until its decline reaches a constant rate, which may take up to one week.

4 Simultaneously 24-hour urinary and faecal radioactivities are determined and the percentage of retained radioactivity is calculated by subtracting the cumulative excreted value from 100% (Fig. 1).

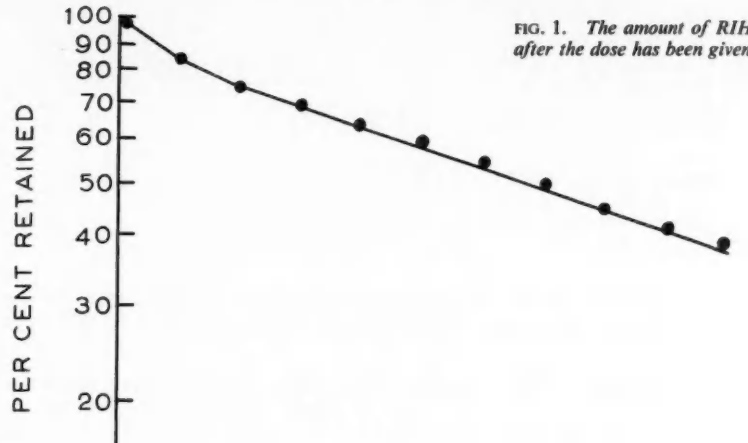

FIG. 1. *The amount of RIHSA left in the body each day after the dose has been given (Case 11).*

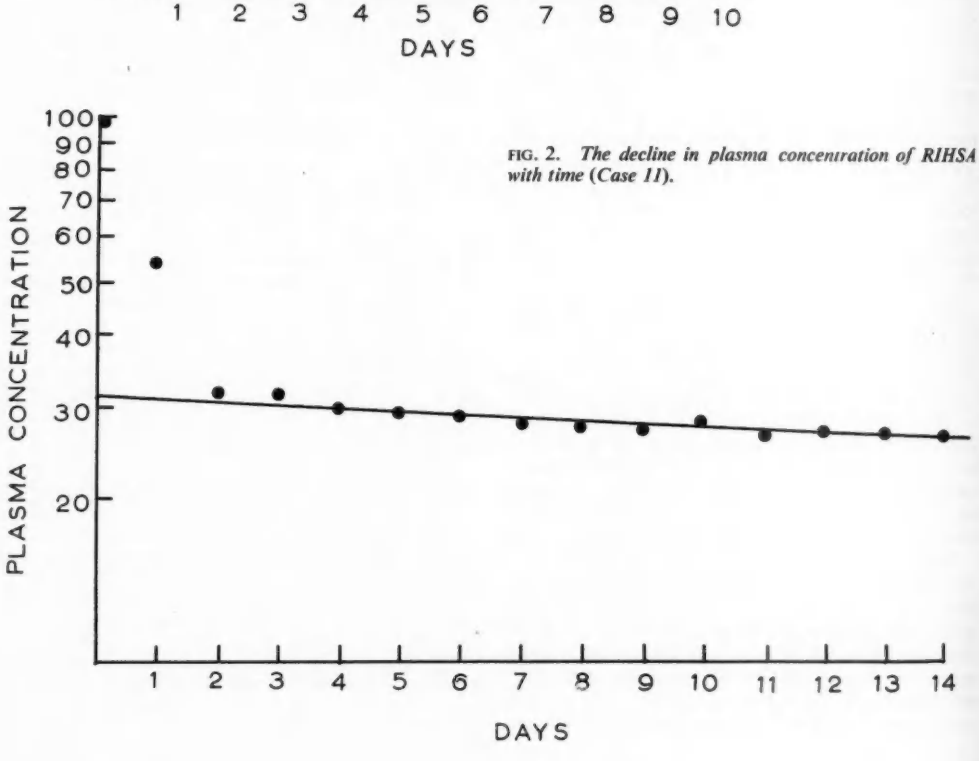

FIG. 2. *The decline in plasma concentration of RIHSA with time (Case 11).*

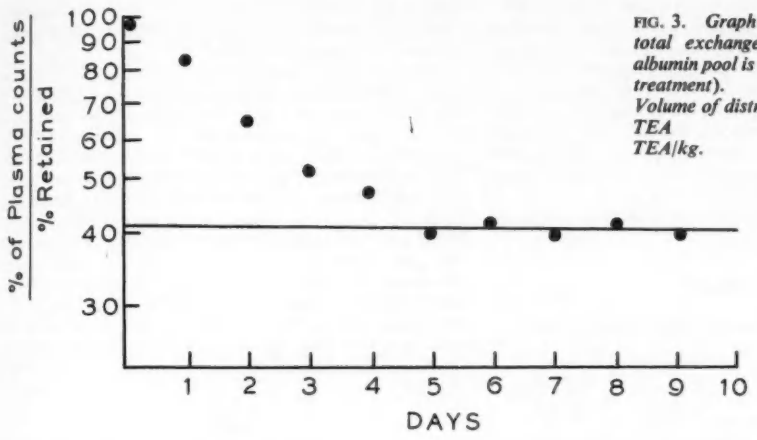

FIG. 3. *Graph demonstrating how the total exchangeable albumin (TEA) or albumin pool is calculated (Case 15 before treatment).*
Volume of distribution = 622 ml.
TEA = 180·5 g.
TEA/kg. = 3·5 g./kg.

5 The activity falls off rapidly at first (Fig. 2) due to dilution in the extravascular pool and destruction of labile molecules.

6 After the initial rapid decay the ratio of the plasma activity on any day (expressed as a percentage of the initial plasma activity) to the percentage retained on that day becomes a constant figure at the point when the decline in the plasma is equal in its rate to the urinary excretion (Fig. 3). At this stage the factor of extravascular dilution is constant. The product of this final dilution and the total plasma concentration of albumin gives the total exchangeable albumin (TEA), or albumin pool.

To determine the turnover of the RIHSA per day, the urinary excretion has to be corrected for the decay in the plasma by plotting the ratio of the urinary excretion in any given day against the percentage retained in the body on that day. This ratio becomes a constant factor and indicates the percentage albumin turnover per day (Fig. 4).

BIOLOGICAL HALF-LIFE This is calculated by extrapolating the slope of plasma radioactivity after it shows a constant rate of fall, to zero time (Fig. 2). The number of days required to reduce the activity to half the value at the intersection is the biological half-life ($T_{\frac{1}{2}}$).

^{131}I-PVP EXCRETION The PVP obtained from Dr. R. S. Gordon was dialysed to remove free iodide before use. The subjects were given 10μc. of ^{131}I-PVP intravenously and the amount excreted in the faeces was estimated for six days.

METHOD FOR STUDYING PROTEIN EXCRETION IN GASTROINTESTINAL TRACT QUANTITATIVELY USING RIHSA We first demonstrated the excretion of RIHSA in the gastrointestinal tract by intubating three patients who had received 30 μc. of RIHSA intravenously and collecting the jejunal secretions from 22 cm. beyond the duodenojejunal flexure. Patients investigated were (1) a patient with quiescent ulcerative colitis (Case 7); (2) a patient with Crohn's disease in a phase of remission (Case 9); (3) a person under investigation with complaints of abdominal distension but with no demonstrable abnor-

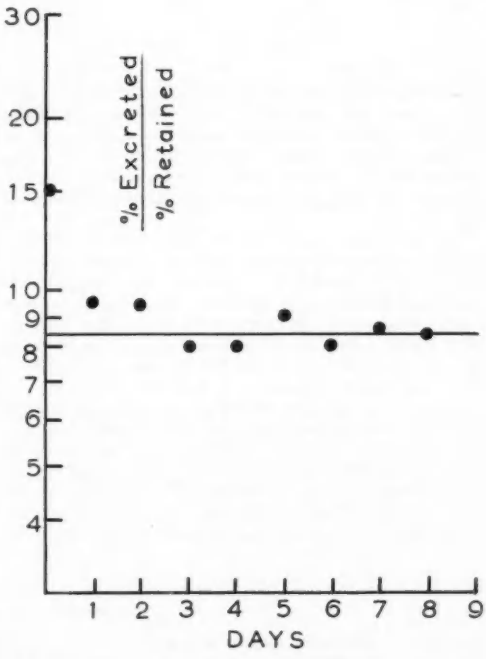

FIG. 4. *The percentage of albumin turned over in one day (Case 15 before treatment).*
Average daily turnover = 8·4%.
Albumin turned over per day = 15·0 g.

mality of the gastrointestinal tract (Case D.H.). Cases 7 and D.H. were thought to have normal small intestinal function. Case 9 had a normal RIHSA turnover during this study. Jejunal juice was collected in vessels containing 10% trichloracetic acid solution (TCA) and in empty ones. The latter were incubated for 20 minutes at 37°C. to allow intestinal enzymes to act on the RIHSA. The precipitate was separated and the supernatant fluid treated with potassium iodide and potassium iodate to liberate iodine which was extracted with chloroform.

The results (Table I) showed that initially only 4 to 5% of the activity was in the form of free iodides, the rest being bound to protein or in a non-ionic form. The digested products contained more free iodide and no protein. Similar results were obtained by Holman, Nickel, and Sleisenger (1959) who showed by electrophoresis that the protein-bound radioactivity resided in the albumin.

TABLE I

PARTITION OF RADIOACTIVITY IN JEJUNAL JUICE

Case No.	*TCA Precipitate (%)*	*Supernatant*	
		Non-Ionic Iodine (%)	*Free Iodide (%)*
Jejunal juice ± trichloracetic acid (TCA)			
7	56·5	38·6	4·9
9	60·1	34·8	5·1
D.H.	58·4	35·0	4·6
Jejunal juice incubated at 37° for 20 minutes			
7	Nil	76·1	23·9
9	Nil	82·6	17·4
D.H.	Nil	80·5	19·5

Having shown that RIHSA was excreted in the gastrointestinal tract and was subsequently digested we then studied the efficiency of Amberlite resin IRA-400 (Cl) (analytical grade) as an agent for absorbing these products of digestion. The resin, in the ratio one to nine by weight, was added to an aliquot of jejunal juice after incubation as previously described. It took up about 80% of the radioactivity (Cases 7, 9, and D.H., Table II).

To study the recovery of RIHSA *in vivo* using Amberlite, 10 μc. of RIHSA was given orally with the Amberlite. The dose of Amberlite was increased in successive experiments and 5 g. four hourly was found to be optimal and resulted in about 80% recovery of the radioactivity in the faeces (Cases A, B, and C, Table II).

In order to determine the excretion of RIHSA in the gut in our subjects we gave them 5 g. of the Amberlite resin four hourly by mouth. The 24-hour faecal radioactivity was determined daily.

The amount of radioactivity excreted in the faeces on a given day depends on the concentration of RIHSA in the plasma on that day. Hence the figure for faecal radioactivity on any particular day must be corrected according to the activity present in the plasma on that day, expressed as a percentage of the activity present on the day the dose of RIHSA was given (the 'percentage radioactivity'). Now faecal activity on one day does not necessarily correspond to the radioactivity excreted into the gut on the previous day because of variations in intestinal motility. We therefore made the correction in the following manner. The total radioactivity was measured in the faeces collected over a four-day period and the mean daily activity was multiplied by the mean 'percentage radioactivity' in the plasma for the same period.

RESULTS

ALBUMIN TURNOVER The biological half-life ($T_{\frac{1}{2}}$) of RIHSA in normal subjects was found by Berson *et al.* (1953) to be always above 12·5 days with an average range of 17 to 20 days. The daily turnover was found to be less than 5·5% per day.

The patients we studied could be placed in four groups and are thus shown in Table III. The $T_{\frac{1}{2}}$ and the daily breakdown in the three control subjects (Cases 1, 2, and 3) in Group I and in the four patients (Cases 4, 5, 6, and 7) with inactive gastrointestinal disorders in Group II, were within normal limits.

Four patients in Group III (Cases 9, 14, 15, and 16) had a markedly accelerated albumin turnover with a $T_{\frac{1}{2}}$ respectively of 3·8, 5·1, 5·5, and 3·5 days. Hypoproteinaemia and oedema were absent in these at the time of study. Four other patients who had hypoproteinaemia and oedema at the time of study (Cases 9, 10, 15, and 17) with a similarly short $T_{\frac{1}{2}}$ were placed separately in Group IV.

The findings in two patients with chronic small bowel obstruction offered an interesting contrast. The $T_{\frac{1}{2}}$ in Case 8, suffering from relatively inactive Crohn's disease, was shortened to 7·2 days, but in Case 11, suffering from non-inflammatory obstruction due to endometriosis, it was normal (22 days). The shortened $T_{\frac{1}{2}}$ in Case 8 could be explained only partly by increased intestinal loss because the

TABLE II

RECOVERY OF RIHSA WITH AMBERLITE

Case No.	*Jejunal Juice/Amberlite Ratio 9/1 (% Recovery)*	*Case No.*	*Dose*	*% Recovery in Faeces*		
In vitro		*In vivo*				
7	85	A	5 g. six hourly	55	63	65
9	79	B	5 g. four hourly	79	74	84
D.H	89	C	5 g. two hourly	80	76	82

TABLE III

PLASMA ALBUMIN LEVELS AND BIOLOGICAL HALF-LIFE ($T_{\frac{1}{2}}$) AND DAILY TURNOVER OF RIHSA IN PATIENTS STUDIED

Case No.	*Clinical State*	*Sex*	*Age*	*Plasma Albumin (g./100 ml.)*	*$T_{\frac{1}{2}}$ (days)*	*Daily Turnover (%)*
Group I: Control subjects						
1	Convalescent myocardial infarction without bowel disturbance	M	65	4·1	17·0	4·1
2		M	55	3·9	22·0	3·1
3		M	47	4·0	20·0	3·2
Group II: Patients with inactive gastrointestinal disorders						
4	Partial gastrectomy	F	49	3·1	17·0	4·1
5	Asymptomatic quiescent ulcerative colitis	F	36	3·8	12·0	5·7
6		F	29	3·8	28·0	2·4
7		M	62	3·7	14·0	5·0
Group III: Patients with gastrointestinal disease but without hypoproteinaemia or oedema						
8	Crohn's disease, before resection and presenting mainly as intestinal obstruction, the E.S.R. being normal	M	26	3·6	7·0	9·6
9	Crohn's disease, in remission	F	46	4·1	3·8	18·2
11	Chronic small intestinal obstruction due to endometriosis	F	48	3·9	22·0	3·1
12	Steatorrhoea after partial gastrectomy	M	66	3·1	6·0	11·5
13	Diarrhoea with pleural effusion (undiagnosed)	F	64	3·1	13·0	5·4
14	Gluten enteropathy (pre-treatment)	M	16	3·6	5·1	13·0
15	Idiopathic steatorrhoea, after partial recovery	F	26	3·9	5·5	13·6
16	Crohn's disease, in relapse	F	39	4·0	3·5	20·5
Group IV: Patients with gastrointestinal disease and with hypoproteinaemia and oedema						
9	Crohn's disease, when active	F	46	2·7	3·5	20·6
10	Crohn's disease, before resection	F	37	2·5	2·8	24·5
15	Idiopathic steatorrhoea, before treatment	F	26	2·9	5·1	13·6
17	Tuberculous strictures (healed) of small intestine	F	37	2·8	3·2	21·0

faecal excretion of RIHSA was only a little above normal (3·4%). The turnover of RIHSA in these patients was respectively 9·6% and 3·1%.

EXCRETION OF ^{131}I-PVP The PVP excretion in three control subjects (Table IV) was below 1·5% of the dose, the upper limit of normal suggested by Gordon (1959).

In four patients with, and in three without, oedema the results were all within the normal range (Table IV). In these patients the PVP method did not indicate any gastrointestinal protein loss although the RIHSA $T_{\frac{1}{2}}$ in all was greatly shortened. However, in three of the four patients with oedema a little more PVP was lost than from those without oedema, although the loss was still within the normal range.

STUDY OF FAECAL RADIOACTIVITY USING RIHSA AND AMBERLITE IRA-400 The results of the excretion of RIHSA with Amberlite suggested that our patients could be regrouped as follows:

1 Control subjects excreted 1·7 to 1·9% of the radioiodine daily (Table V) corresponding to about 5 g. of albumin per day (calculated from the total exchangeable albumin). The daily turnover of

TABLE V

ALBUMIN TURNOVER AND FAECAL EXCRETION OF RIHSA IN NORMAL SUBJECTS (GROUP I OF TABLE III)

Case No.	*Clinical State*	*$T_{\frac{1}{2}}$ (days)*	*Daily Turnover (%)*	*Daily Excretion with Amberlite (% of dose)*
1	Convalescent myocardial infarction	17·0	4·1	1·7
2		22·0	3·1	1·8
3		20·0	3·2	1·9

RIHSA amounted to 3·1 to 4·1% of RIHSA per day corresponding to a turnover of 9·4 to 12·3 g. of albumin per day. Similar results were seen in the patients whose gastrointestinal tracts were functionally normal at the time of study (Table VI).

TABLE IV

^{131}I-PVP EXCRETION OVER SIX DAYS AFTER AN INTRAVENOUS DOSE

Case No.	*% of Dose*	*Case No.*	*% of Dose of ^{131}I-PVP*	*$T_{\frac{1}{2}}$ of RIHSA*	*Case No.*	*% of Dose of ^{131}I-PVP*	*$T_{\frac{1}{2}}$ of RIHSA*
In three control subjects		*Patients without oedema*			*Patients with oedema*		
1	0·86	9	0·67	3·8	9	0·98	3·5
2	0·51	14	0·47	6·0	10	0·38	2·8
3	1·20	15	0·87	5·5	15	1·1	5·1
					17	1·3	3·2

TABLE VI

ALBUMIN TURNOVER AND FAECAL EXCRETION OF RIHSA IN PATIENTS WITH INACTIVE GASTROINTESTINAL DISEASE (GROUP II OF TABLE III)

Case No.	Clinical State	$T\frac{1}{2}$ (days)	Daily Turnover (%)	Daily Excretion with Amberlite (% of dose)
4	Partial gastrectomy	17·0	4·1	1·9
5	Symptomless, quiescent ulcerative colitis	12·0	5·7	1·8
6		28·0	2·4	1·9
7		14·0	5·0	1·6

2 The second group was composed of the four patients with non-specific intestinal disorders (Table VII). The excretion of radioiodine into the gastrointestinal tract in these patients amounted to 2·1 to 4·8% daily, corresponding to a loss of about 7 g. albumin per day.

3 The third group was composed of patients with hypoalbuminaemia and oedema (Table VIII). The excretion of RIHSA was 12·9 to 18·9% daily corresponding to a loss of about 22 g. of albumin per day.

4 The fourth group (Table IX) comprised patients in whom hypoproteinaemia and oedema were absent but in whom the endogenous protein breakdown of RIHSA was 7·7 to 20·5% daily which markedly exceeded the amount excreted in the gastrointestinal tract. Gastrointestinal excretion in this group (2·4 to 2·5%) was the same as in the patients in Group 2.

The effect of a gluten-free diet in a patient with gluten enteropathy (Case 14) and of antibiotics for three weeks in a patient with idiopathic steatorrhoea (Case 15) is shown in Table X.

DISCUSSION

The method described for the study of the excretion of protein into the gastrointestinal tract has the advantages of using radioiodinated albumin as a tracer. Radio-iodinated human serum albumin and native albumin are closely related to each other chemically and physically.

That this method actually measures the albumin excreted and not dissociated radioiodine is indicated by the following evidence:

Most of the radioactivity excreted was at first either protein-bound or in a non-ionic form (found to be mono-iodo-tyrosine by Parkins, Dimitriadou, and Booth, 1960). The free iodide constituted only 4·5% of the radioactivity.

This radioactivity was taken up by Amberlite to the extent of 80%. Hence the faecal radioactivity after the administration of this resin was quantitatively representative of the excreted albumin.

The study of a group of subjects with rapid turnover of RIHSA in the plasma without increased activity in the faeces indicates that the excreted radioactivity is not secondary to the shortened

TABLE VII

ALBUMIN TURNOVER AND FAECAL EXCRETION OF RIHSA IN PATIENTS WITH NON-SPECIFIC GASTROINTESTINAL DISORDERS (PART OF GROUP III OF TABLE III)

Case No.	Condition	$T\frac{1}{2}$ (days)	Daily Albumin Turnover (%)	Daily Excretion in Gastrointestinal Tract	
				RIHSA (%)	Albumin[1] (g.)
8	Crohn's disease, small intestinal obstruction	7·2	9·6	3·4	5·7
11	Endometriosis, small intestinal obstruction	22·0	3·1	2·2	7·6
12	Partial gastrectomy steatorrhoea	6·0	11·5	4·8	8·4
13	Diarrhoea	13·0	5·4	2·1	5·4

[1]Calculated from the total exchangeable albumin (TEA).

TABLE VIII

ALBUMIN TURNOVER AND FAECAL EXCRETION OF RIHSA IN PATIENTS WITH HYPOPROTEINAEMIA AND OEDEMA (GROUP IV OF TABLE III)

Case No.	Condition	$T\frac{1}{2}$ (days)	Daily Albumin Turnover (%)	Daily Excretion in Gastrointestinal Tract	
				RIHSA (%)	Albumin[1] (g.)
9	Crohn's disease (active)	3·5	20·6	18·9	17·8
10	Crohn's disease (active)	2·8	24·5	15·5	15·2
15	Idiopathic steatorrhoea (active)	5·1	13·6	17·2	30·9
17	Tuberculous strictures of small intestine	3·2	21·0	12·9	22·5

[1]Calculated from the TEA.

TABLE IX

ALBUMIN TURNOVER AND FAECAL EXCRETION OF RIHSA IN PATIENTS WITH SMALL BOWEL DISEASE WITHOUT HYPOPROTEINAEMIA OR OEDEMA (PART OF GROUP III OF TABLE III)

Case No.	*Condition*	*$T_{\frac{1}{2}}$ (days)*	*Daily Albumin Turnover (%)*	*Daily Excretion in Gastrointestinal Tract*	
				RIHSA (%)	*Albumin[1] (g.)*
9	Crohn's disease (in remission)	3·8	18·2	2·4	5·1
14	Gluten enteropathy	5·0	7·7	2·5	4·7
15	Idiopathic steatorrhoea	5·5	12·6	2·4	5·4
16	Crohn's disease (in remission)	3·5	20·5	2·4	5·2

[1]Calculated from the TEA.

TABLE X

EFFECT OF TREATMENT IN STEATORRHOEA ON THE EXCRETION OF RIHSA INTO THE GUT

Case No.	*Disease Active*			*In Remission*		
	$T_{\frac{1}{2}}$ (days)	*Albumin Pool (g.)*	*RIHSA Excretion (% of dose)*	*$T_{\frac{1}{2}}$ (days)*	*Albumin Pool (g.)*	*RIHSA Excretion (% of dose)*
14	5·1	180	17·2	5·5	292	2·4
15	9·0	100	10·7	10·1	190	2·5

survival of RIHSA in the plasma, with subsequent excretion of the ^{131}I so liberated.

ALBUMIN TURNOVER STUDIES These studies indicated that there was no relation between the $T_{\frac{1}{2}}$ and the presence of hypoproteinaemia and oedema in patients with small bowel disease. The $T_{\frac{1}{2}}$ in patients without oedema was slightly greater than in four with oedema due to hypoproteinaemia.

There seem to be at least two possible explanations for this discrepancy:—

1 There are two types of persons: those who have a reserve of regenerative capacity for the manufacture of plasma proteins to meet the needs of increased destruction, and those in whom this reserve is lacking. We have no evidence for this explanation.

2 In cases of hypoproteinaemia where protein is being lost through the gastrointestinal tract there may be an inability adequately to reabsorb and use the products of digestion in the presence of enteropathy. The plasma albumin seems to be in equilibrium at a lower level. At this level the absolute amounts turned over will be lower than at normal levels. For example, the albumin pool in Case 9 (when active) was 98 g. and her daily turnover was 20·6%. Hence albumin turned over in a day

$$= \frac{20{\cdot}6}{100} \times 98 = 20{\cdot}2 \text{ g.}$$

If her pool was 300 g. the daily turnover would be

$$\frac{20{\cdot}6}{100} \times 300 = 61{\cdot}8 \text{ g.}$$

Thus if she was unable to re-use the proteins excreted she would have to 'set' her albumin at a lower level.

STUDY OF FAECAL RADIOACTIVITY USING RIHSA From the study of control subjects and of patients with functionally normal gastrointestinal tracts, it was seen that about 1·8% of the albumin was excreted daily and a similar amount was broken down endogenously. This indicates that albumin is normally lost from the albumin pool in two ways: half by endogenous breakdown and half in the gastrointestinal tract.

This dual mechanism was found in most of the patients. However, in contradistinction to the control subjects, endogenous breakdown of RIHSA in most of the patients was greater than intestinal loss. This was particularly obvious in the patients with disease of the small bowel but without hypoproteinaemia or oedema (Table IX).

COMPARISON OF ^{131}I-PVP AND RIHSA In patients with hypoproteinaemia and oedema there was a marked increase in the excretion of RIHSA whereas the PVP excretion was normal and did not reveal the true state of affairs in these patients. This lack of correlation is shown in Table XI.

The fact that the excretion of RIHSA may sometimes be as high as 4·8% per day without a fall in plasma albumin (Table VII) suggests that RIHSA excretion must be above about 5% per day before hypoproteinaemia will appear.

EFFECT OF THERAPY There was a marked decrease in RIHSA excretion in two patients with steatorrhoea after treatment with a gluten-free diet (Case 14) and with antibiotics (Case 15). In both the excretion was

TABLE XI

CORRELATION OF RIHSA EXCRETION AND ^{131}I-PVP EXCRETION

Case No.	*Condition*	$T_{\frac{1}{2}}$ *(days)*	*Excretion of RIHSA into Gastrointestinal Tract (% of dose)*	^{131}*I-PVP Excretion (% of dose)*
15	Idiopathic steatorrhoea (active)	5·1	17·2	1·1
15	Idiopathic steatorrhoea (partially treated)	5·5	2·4	0·87
9	Crohn's disease (active)	3·5	18·9	0·98
10	Crohn's disease (active)	2·8	15·5	0·38
17	Tuberculous strictures of small intestine	3·2	12·9	1·3

reduced to within the range found in non-specific bowel conditions such as obstruction.

Patients with active idiopathic steatorrhoea lose considerable amounts of RIHSA in the faeces (Cases 14 and 15). The use of RIHSA and Amberlite described here may be a fruitful method for studying the effect of various modes of treatment on such small bowel disorders.

SUMMARY

Methods of studying gastrointestinal protein loss are reviewed. The advantages of RIHSA and the disadvantages of ^{131}I-PVP are discussed.

Three control subjects and 15 patients with different intestinal disorders were studied (*a*) using intravenous RIHSA to estimate the albumin turnover, (*b*) measuring faecal radioactivity after intravenous injection of ^{131}I-PVP, and (*c*) measuring the faecal excretion of RIHSA using oral Amberlite resin IRA-400 (Cl.).

Jejunal intubation demonstrated that intact RIHSA was excreted into the jejunal lumen. The Amberlite resin was found to be capable of absorbing about 80% of RIHSA in the gut.

Albumin turnover studies indicated that there was no relation between the $T_{\frac{1}{2}}$ and the presence of hypoproteinaemia and oedema. The possible reasons for this are discussed.

The faecal excretion of ^{131}I-PVP was normal in our patients with hypoproteinaemia.

Studies of the excretion of injected RIHSA indicated that the turnover of albumin in control subjects was dependent half on endogenous albumin breakdown, and half on excretion and digestion of body albumin in the intestine. The actual amount of RIHSA excreted into the gastrointestinal tract per day was less than 2% of the injected dose.

Patients with inactive or non-specific bowel conditions excreted up to about 4% of the injected dose of RIHSA. This figure increased to about 9% in patients with active small bowel disease associated with hypoproteinaemia and oedema. Patients with small bowel disease but no hypoproteinaemia or oedema excreted, with one exception, under 4% of the injected dose of RIHSA daily.

In two patients with steatorrhoea the daily RIHSA excretion after treatment fell from 10·7 and 17·2% respectively to about 2·5% and the albumin pools increased by about 60 to 100% although the $T_{\frac{1}{2}}$ remained abnormally short in both.

We are grateful to Dr. R. S. Gordon, National Heart Institute, Bethesda, Maryland, U.S.A., for supplying the ^{131}I-PVP used, and to Dr. J. R. Mallard, Hammersmith Hospital, for helping us to obtain it.

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REFERENCES

Berson, S. A., Yalow, R. S., Schreiber, S. S., and Post, J. (1953). Tracer experiments with I^{131}-labelled human serum albumin: distribution and degradation studies. *J. clin. Invest.*, **32**, 746-768.

Gordon, R. S. (1959). Exudative enteropathy. Abnormal permeability of the gastrointestinal tract demonstrable with labelled polyvinylpyrrolidone. *Lancet*, **1**, 325-326.

Gross, P. A. M., Embree, L. J., Bally, P., Shipp, J. C., and Thorn, G. W. (1960). Hypoalbuminemia (with anasarca) due to hypercatabolism, serum protein exudation into the gastrointestinal tract, increased capillary permeability and hypoanabolism. *Amer. J. Med.*, **29**, 386-404.

Holman, H., Nickel, W. F. Jr., and Sleisenger, M. H. (1959). Hypoproteinemia antedating intestinal lesions, and possibly due to excessive serum protein loss into the intestine. *Ibid*, **27**, 963-975.

McFarlane, A. S. (1956). Labelling of plasma proteins with radioactive iodine. *Biochem. J.*, **62**, 135.

Parkins, R. A., Dimitriadou, A., and Booth, C. C. (1960). The rates and sites of absorption of ^{131}I-labelled albumin and sodium ^{131}I in the rat. *Clin. Sci.*, **19**, 595-604.

Schwartz, M., and Jarnum, S. (1959). Gastrointestinal protein loss in idiopathic (hypercatabolic) hypoproteinaemia. *Lancet*, **1**, 327-330.

——, and Thomsen, B. (1957). Idiopathic or hypercatabolic hypoproteinaemia. Cases examined by ^{131}I-labelled albumin. *Brit. med. J.*, **1**, 14-17.

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The stability of the radiotriolein bond in intestinal secretions

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SYNOPSIS The radiotriolein test has been reported to be unreliable in the diagnosis of steatorrhoea. A possible explanation is provided by these studies in which labelled triolein was incubated in human gastric juice, duodenal juice, and faeces; the bond was not stable on duodenal juice.

Radio-iodinated triolein (RIT) has been widely used in the investigation of intestinal malabsorption. The method is essentially a tracer test in which a known dose of RIT is given by mouth and the percentage of the administered isotope appearing in the plasma or excreted in the faeces is measured. This provides an assessment of fat digestion and absorption by the alimentary tract.

The reliability of the method was not seriously questioned until Pimparkar, Tulsky, Kalser, and Bockus (1960) reported a large series of cases tested by concurrent faecal chemical fat and faecal RIT measurements. Taking a daily faecal chemical fat excretion of over 7·0 g. as the criterion for steatorrhoea, they found that the RIT test failed to diagnose the condition in 25% of cases. In a personal series of 33 patients found to have steatorrhoea by the faecal chemical fat excretion, the RIT test failed in 50% (Cox, 1961). A possible explanation of these findings is that the radio-iodine label is unstable in the alimentary tract. Since the metabolic pathway of iodine differs from that of fat, instability of the RIT bond would clearly vitiate the test.

Experimental testing of the RIT bond in intestinal secretions has been reported by Beres, Wenger, and Kirsner (1957) and by Balint, Pendower, and Ramsey (1960). The former group incubated RIT with gastric juice, pancreatic juice, and bile for one hour, and, by measuring the activity in the aqueous fraction after extraction with petroleum ether, they concluded that the bond was stable over the period of the experiment. Using a similar method, Balint and his colleagues incubated RIT in a wider range of media. Instability was not detected during four hours' incubation in gastric or duodenal secretions, but a small amount of free radio-iodine was liberated in faeces over a two-day period of incubation.

The use of an anion exchange resin to determine the percentage of free I^{131} in radio-iodinated lipids (Cox and Hinchliffe, 1961) offers a simple means of repeated testing of the stability of the bond, permits the behaviour of the bond to be followed over a long period of time, and allows a comparison of the characteristics of different batches of RIT. This paper reports the results of such a study.

METHOD

The experiments were conducted in the following way. The batch of RIT under test was emulsified with Tween 80 and water, and incubated with controls and intestinal secretions. Water at 20°C. and 37°C. served as controls. The intestinal secretions studied were gastric juice, duodenal juice, and faeces, each incubated at 37°C. Samples were taken from the incubation mixtures at intervals of 0, four, eight, 24, 48, 72, and 96 hours and tested for the amount of free I^{131} present. A rise in the percentage of free I^{131} was taken to indicate instability of the bond under the conditions of the test.

MATERIALS

RADIOTRIOLEIN Radioiodine (I^{131}) in two different chemical forms was used to label triolein.

Triolein labelled with I^{131} as elementary iodine The fat in ethereal solution was mixed with elementary I^{131} in ether. After allowing 48 hours for iodination, any remaining free I^{131} was removed by washing three times with a solution of sodium thiosulphate and potassium iodide. This was followed by three washes with distilled water. The ether was evaporated on a water-bath at 50°C.

Triolein labelled with I^{131} as iodine monochloride Iodine monochloride (I^{131} Cl) is generally accepted as the more suitable label. Four batches of triolein, differing according to labelling procedure or source, were selected for test. Brief details of these are given in the following paragraphs. In the description of the results they will be referred to by the letters given here.

A RIT was purchased from the Radiochemical Centre at Amersham. Full details of the labelling procedure are not available; the Hormel Foundation supplies triolein to Amersham.

B Triolein from the Aldrich Chemical Company was labelled by the method of Lubran and Pearson (1958).

C Synthetic triolein from Unilever Ltd. was labelled in the following way. Elementary iodine, liberated from an aqueous solution of NaI^{131} and carrier KI by oxidation with potassium iodate and sulphuric acid, was extracted into carbon tetrachloride and dried on a column of anhydrous sodium sulphate. A solution of chlorine in carbon tetrachloride, dried in the same way, was added dropwise to the I^{131} solution until it turned brown, the colour of iodine monochloride in carbon tetrachloride (Sidgwick, 1950). This iodinating solution was added to triolein dissolved in carbon tetrachloride. After the mixture had been allowed to react in the dark at 20°C. for three hours, any remaining free I^{131} was extracted into a weak solution of sodium thiosulphate and converted to I^{131} Cl in carbon tetrachloride as before, thus making it available for further iodination. After standing overnight, the solution was washed with sodium thiosulphate and potassium iodide solution, followed by water. The carbon tetrachloride was evaporated on a water-bath at 50°C. This two-stage iodination procedure was used in order to increase the percentage tag (Dossett, 1960).

D Triolein from the Aldrich Chemical Company was labelled in the same way as C.

INCUBATION MEDIA Four were used.

1 *Water at 20°C. and 37°C.* These served as controls and to assess the stability of the bond when RIT is stored as an emulsion for the purpose of dosing patients.

2 *Human gastric juice at 37°C.* Specimens were obtained during maximal histamine stimulation from duodenal ulcer patients undergoing routine pre-operative augmented histamine tests (Kay, 1953).

3 *Human duodenal juice at 37°C.* Samples were obtained by duodenal aspiration during secretin and pancreozymin stimulation. A pooled specimen, containing bile and pancreatic juice, was used. No attempt was made to exclude contamination with gastric secretions, since the intention was to mimic as closely as possible the secretions with which ingested RIT comes into contact. Most specimens were obtained from duodenal ulcer patients and their secretions will be referred to as 'hypersecretor' juice. One specimen was from a patient with a Polya partial gastrectomy, who failed to secrete acid on maximal histamine stimulation; his secretions will be referred to as 'achlorhydric' juice.

Duodenal juice was used both untreated and after boiling for 30 minutes; these will be referred to as 'fresh' and 'boiled' juice respectively. When not required for immediate use, the duodenal juice was stored at −40°C. and thawed out slowly at 2°C. immediately before the start of an experiment.

4 *Homogenized human faeces at 37°C.* Specimens were obtained immediately after defaecation from a healthy subject.

RESULTS

STABILITY OF THE BOND IN WATER The I^{131} Cl labelled triolein remained stable in water at 20°C. and 37°C. throughout 96 hours (Table I). By contrast the simple I^{131} label was not completely stable.

STABILITY OF THE BOND IN GASTRIC JUICE Two batches of I^{131} Cl labelled triolein were tested and each showed stability in gastric juice at 37°C. over the 96-hour period of study (Table II).

STABILITY OF THE BOND IN DUODENAL JUICE Since the first few experiments revealed appreciable instability of the bond in duodenal juice, this

TABLE I

STABILITY OF THE RADIOTRIOLEIN BOND IN WATER

At 20°C.		*Free I^{131} as Percentage of Total I^{131}*							*No. of Specimens*
		Hours of Incubation 0	4	8	24	48	72	96	
I^{131} Cl label	A	0·5	1	1	0	8·5	5	2·5	2
	B	2	0	0	9	0	2	5	1
	C	0·7	0·3	2·3	3·1	4·0	0·3	1	3
	D	1	0	2	4	5	10	6	1
	Mean	1	0·3	1·3	4·0	4·4	4·4	3·8	7
Simple I^{131} label		5	8	3	6·5	9·5	13·5	15·5	2
At 37°C.									
I^{131} Cl label	A	7	0	0	0	8	0	2	2
	B	0	0	0	8	0	4	2	1
	C	2	0·3	2	0	2	2·3	2	3
	D	5	0	0	0	0	5	4	1
	Mean	2·9	0·1	0·9	2	1·9	3·3	3	7
Simple I^{131} label		2	4·5	1	9	13	8	12	2

TABLE II

STABILITY OF RADIOTRIOLEIN BOND IN HUMAN GASTRIC JUICE

At 37°C.		*Free I^{131} as Percentage of Total I^{131}*						
		Hours of Incubation						
		0	*4*	*8*	*24*	*48*	*72*	*96*
I^{131} Cl label	A	0	0	0	0	4	0	0
	B	0	1	1	3	0	7	0

medium was tested more extensively. The results of the tests with RIT in fresh and boiled hypersecretor duodenal juice are given in Table III, and Fig. 1 illustrates the rise in the percentage of free I^{131} of each batch in the fresh juice. The findings may be summarized as follows:—

The instability of the I^{131} Cl bond first becomes apparent on 24 hours' incubation in fresh, hypersecretor juice and increases thereafter.

The I^{131} Cl bond is stable in boiled, hypersecretor juice.

All batches of I^{131} Cl labelled triolein behave in the same way in duodenal juice.

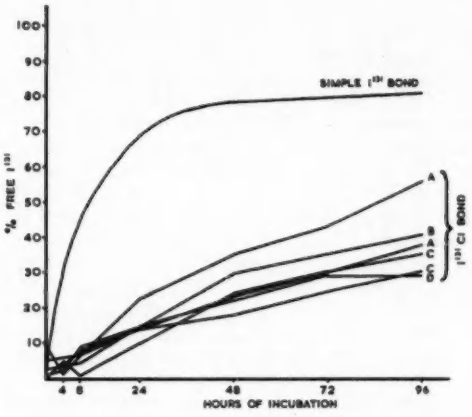

FIG. 1. *Diagram to show the rise in the percentage of free I^{131} in seven batches of radio-iodinated triolein incubated at 37°C. with fresh, hypersecretor duodenal juice over a period of 96 hours.*

TABLE III

STABILITY OF RADIOTRIOLEIN BOND IN DUODENAL SECRETIONS

At 37°C.		*Free I^{131} as Percentage of Total I^{131}*							*No. of Specimens*
		Hours of Incubation							
		0	*4*	*8*	*24*	*48*	*72*	*96*	
Fresh hypersecretor juice									
I^{131} Cl label	A	7	4	7	18	30	36	47	2
	B	3	4	5	15	30	36	41	1
	C	2	4	5	12	21	28	33	2
	D	1	3	9	15	22	29	29	1
	Mean	3·2	3·8	6·5	15	25·8	32·7	35	6
Simple I^{131} label		1	30	46	69	78	80	82	1
Boiled hypersecretor juice									
I^{131} Cl label	A	3·5	0	0	1·5	1	3·5	5	2
	B	4	2	7	6	4	2	3	2
	C	2·5	4	2·5	2·5	4·5	4	9	2
	D	2	5	0	4	7	6	5	1
	Mean	3	2·8	2·4	3·5	4·1	3·9	5·5	7
Simple I^{131} label		5	2	4·5	4·5	24	29	36	2

The simple I^{131} bond shows marked instability after four hours' incubation with fresh, hypersecretor juice.

The simple I^{131} bond is unstable after 48 hours' incubation with boiled hypersecretor juice.

One batch of each of the I^{131} Cl and the simple I^{131} labelled triolein was tested in the achlorhydric duodenal juice. Both bonds were more stable in the fresh achlorhydric juice than in the fresh hypersecretor juice. Each bond behaved in the same way in boiled achlorhydric juice as in boiled hypersecretor juice; the I^{131} Cl bond remained stable over 96 hours of incubation, whereas instability of the simple I^{131} bond appeared after 48 hours' incubation (Fig. 2).

STABILITY OF THE BOND IN FAECES The I^{131} Cl labelled triolein showed a minor degree of in-

TABLE IV

STABILITY OF RADIOTRIOLEIN BOND IN HUMAN FAECES

At 37°C.		*Free I^{131} as Percentage of Total I^{131}*						
		Hours of Incubation						
		0	*4*	*8*	*24*	*48*	*72*	*96*
I^{131} Cl label	A	3	5	2	5	6	7	8
	B	0	7	1	3	10	12	9

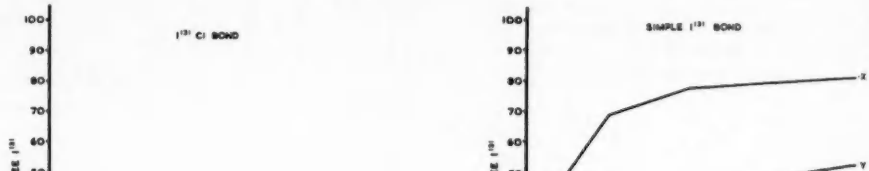

FIG. 2. *The stability of radio-iodinated triolein in hypersecretor duodenal juice (X) and achlorhydric duodenal juice (Y). The unbroken lines represent experiments with samples of the fresh juice and the broken lines experiments with the boiled juice.*

stability after 96 hours' incubation in faeces at 37°C. (Table IV).

DISCUSSION

The conventional fat balance is generally held to be a difficult and tedious investigation. The introduction of the RIT test raised hopes that a simpler and more reliable means of diagnosing steatorrhoea had been found, and early reports appeared to justify this optimism. More recently, however, evidence has accumulated to show that the method is less sensitive than the fat balance in the diagnosis of steatorrhoea. Several groups of workers have found that the RIT test fails to diagnose steatorrhoea in a substantial number of cases in which the condition has been confirmed by concurrent faecal chemical fat determinations (Pimparkar *et al.*, 1960; Vaughan Jones, 1960; Cox, 1961). While technical errors, such as incomplete stool collection, may account for the failure of RIT in some cases, it is unlikely that these provide the complete explanation.

The RIT test relies for its validity on at least two fundamental assumptions which require verification before the method can be accepted. First, ingested RIT can at best be expected to follow the fate of dietary fat in the alimentary tract, and it is therefore fundamental to the RIT test that faecal chemical fat should be of predominantly dietary origin. However, Cooke, Thomas, Mangall, and Cross (1953) have reported patients with steatorrhoea in whom the faecal chemical fat excretion was appreciably higher than the dietary fat intake. In these cases, the lipid content of the stool may originate in part from the bile (McClure, Huntsinger, and Fernald, 1934), synthesis of fats by the cells of the intestinal mucosa (Clark and Hübscher, 1960), active fat secretion by the bowel mucosa (Lewis and Partin, 1954), or by bacterial synthesis (Sammons, Vaughan, and Frazer, 1956). In patients with a normal faecal RIT excretion, a high faecal chemical fat excretion might be explained if the faecal lipids were of endogenous origin and not derived from unabsorbed dietary fat. The second fundamental assumption is that the radio-iodine label remains firmly attached to triolein in the alimentary tract; appreciable instability of the bond would annul any value that RIT might have as a diagnostic or research tool. The studies reported in this paper are concerned with this facet of the problem and have been designed to extend the observations of previous workers who found that the bond was stable over a relatively short period of time in the intestinal secretions they used (Beres *et al.*, 1957; Balint *et al.*, 1960).

Triolein labelled with radio-iodine in the simple elementary form has been used recently in clinical studies by Siurala, Lamberg, Turula, and Härkönen (1959). Our experiments with this material showed that 46% of the radio-iodine is split off after four hours' contact with fresh duodenal juice and that prolonged incubation resulted in the liberation of increasing amounts of the radio-isotope. Boiling the duodenal juice greatly diminished its bond-splitting property. In the water controls, only a minor degree of instability of the bond was detected. These findings support the view of Veall and Vetter (1958) that the simple radio-iodine label is unstable in the alimentary tract and therefore unsuitable for clinical use.

Triolein labelled with I^{131} as iodine monochloride is the more widely used material and has therefore been studied more extensively in these experiments, in order to compare the characteristics of different batches of RIT and to determine its stability in a wider range of intestinal secretions.

Thirty-six experiments were carried out using different incubation media and different batches of RIT. Each batch behaved similarly in a given set of conditions. This may be taken as evidence that the stability of the I^{131} Cl bond is not materially affected by varying the method of the labelling procedure and that each type of triolein used for labelling had similar properties.

Incubation for 96 hours showed that the I^{131} Cl label is stable in water at 20°C. and 37°C. This confirms the findings of Chears, McCraw, Tyor, and Ruffin (1958), and demonstrates that RIT for clinical use may be stored as an emulsion. In gastric juice free I^{131} was not released from RIT, indicating that the bond is stable in the stomach. In faeces, however, a small rise in the percentage of free I^{131} was detected over the four-day incubation period. This is in keeping with the finding of Balint *et al.* (1960), but the degree of instability is so small that it seems unlikely to invalidate the clinical use of RIT.

The short period of incubation in duodenal juice used by other workers did not reveal any instability of the I^{131} Cl bond. In the experiments reported here it was found that extending the period of contact with duodenal juice caused an appreciable rise in the percentage of free I^{131}. In fresh duodenal juice, 15% of the I^{131} Cl label was split off after 24 hours' incubation and 35% at the end of 96 hours. The same juice, when boiled, had no effect on the bond, suggesting that the bond-splitting property of duodenal juice rests in its enzyme content.

The relevance of these findings to the use of RIT in the investigation of malabsorption is difficult to assess. The view may be held that the clinical use of the material is not jeopardized, since ingested RIT does not stay in the small intestine for the period of 24 hours required to demonstrate instability of the bond *in vitro*. However, these experiments suggest that the enzymes of duodenal juice are responsible for the breakdown of the bond, and Lagerlöf (1942) has shown that the lipolytic activity of duodenal juice falls by as much as 70% when kept at 37° C. for a period of 30 minutes. Whether this decline in activity occurs as rapidly in the alimentary tract is uncertain. If the enzyme activity of duodenal juice is maintained throughout the small intestine, it is possible that its bond-splitting potency is greater in the bowel than in the test-tube. If this is so, the instability of the bond may reduce the reliability of the RIT test in the assessment of malabsorption.

If the breakdown of the radio-iodine linkage accounts for the failure of RIT to diagnose steatorrhoea in some cases, its success in others remains to be explained. In this connexion, the finding that duodenal juice from an achlorhydric, post-gastrectomy patient had markedly reduced bond-splitting properties was of considerable interest. It may be that biliary and pancreatic secretions from different subjects differ in their power to split the bond and that failure to diagnose steatorrhoea occurs where bond-splitting potency is high. On the evidence available, it would be speculative to carry the argument further than the statement that the behaviour of RIT in the alimentary tract may vary from patient to patient. These results *in vitro* can best be interpreted in the light of studies *in vivo* which are now in progress.

SUMMARY

The purpose of the study was to investigate the stability of the radio-iodinated triolein bond in intestinal secretions.

Labelled triolein was incubated in gastric juice, duodenal juice, and faeces. At intervals over a period of 96 hours, samples were taken from the incubation mixtures and the percentage of liberated I^{131} determined.

The simple I^{131} label was slightly unstable in water and very strikingly unstable in duodenal juice. By boiling the duodenal juice, its bond-splitting potency was reduced.

The I^{131} Cl label was stable in water and gastric juice. A minor degree of instability in faeces was detected. In duodenal juice, instability of the bond first became apparent after 24 hours' incubation. No free I^{131} appeared when the duodenal juice was boiled.

The duodenal juice from an achlorhydric patient affected the bond less than did the juice from duodenal ulcer patients.

These results provide one possible explanation for the reported unreliability of the radiotriolein test.

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REFERENCES

Balint, J., Pendower, J., and Ramsey, N. W. (1960). The stability of radio-iodinated olive oil. *Clin. Sci.*, **19**, 321-325.

Beres, P., Wenger, J., and Kirsner, J. B. (1957). The use of I^{131} triolein in the study of absorptive disorders in man. *Gastroenterology*, **32**, 1-16.

Chears, W. C., McCraw, B. H., Tyor, M. P., and Ruffin, J. M. (1958). The I^{131} labeled triolein absorption test; reproducibility and factors affecting blood levels. *Sth. med. J.* (*Bgham, Ala.*), **51**, 433-437.

Clark, B., and Hübscher, G. (1960). Biosynthesis of glycerides in the mucosa of the small intestine. *Nature* (*Lond.*), **185**, 35-37.

Cooke, W. T., Thomas, G., Mangall, D., and Cross, H. (1953). Observations on the faecal excretion of total solids, nitrogen, sodium, potassium, water, and fat in the steatorrhoea syndrome. *Clin. Sci.*, **12**, 223-234.

Cox, A. G. (1961). An assessment of the radio triolein test. In the press.

——, and Hinchliffe, Z. (1961). A method for the determination of free I^{131} in radio-iodinated lipids. In the press.

Dossett, J. A. (1960). Personal communication.

Kay, A. W. (1953). Effect of large doses of histamine on gastric secretion of HCl. *Brit. med. J.*, **2**, 77-80.

Lagerlöf, H. O. (1942). Pancreatic function and pancreatic disease. *Acta med. scand.*, Suppl. 128, pp. 55-61.

Lewis, G. T., and Partin, H. C. (1954). Fecal fat on an essentially fat-free diet. *J. Lab. clin. Med.*, **44**, 91-93.

Lubran, M., and Pearson, J. D. (1958). A screening test for steatorrhoea using I^{131} labelled triolein. *J. clin. Path.*, **11**, 165-169.

McClure, C. W., Huntsinger, M. E., and Fernald, A. T. (1934). The fatty acids of human duodenal bile. *Amer. J. Physiol.*, **107**, 1-12.

Pimparkar, B. D., Tulsky, E. G., Kalser, M. H., and Bockus H. L. (1960). Correlation of radioactive and chemical faecal fat in different malabsorption syndromes. *Brit. med. J.*, **2**, 894-900.

Sammons, H. G., Vaughan, D. J., and Frazer, A. C. (1956). Synthesis of long-chain fats by bacteria isolated from human faeces. *Nature* (*Lond.*), **177**, 237.

Sidgwick, N. V. (1950). *The Chemical Elements and Their Compounds.* Vol. 2, p. 1153. Clarendon Press, Oxford.

Siurala, M., Lamberg, B-A., Turula, K., and Härkönen, P. (1959). The estimation of intestinal fat absorption using I^{131} labelled olive oil. *Ann. Med. exp. Fenn.*, **37**, 424-432.

Vaughan Jones, R. (1960). *Brit. med. J.*, **2**, 1236-1237.

Veall, N., and Vetter, H. (1958). *Radioisotope Techniques in Clinical Research and Diagnosis*, p. 344. Butterworth, London.

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Proctalgia fugax

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SYNOPSIS A personal study of 24 patients suffering from protalgia fugax is reported. It is suggested that sudden vascular congestion analogous to migraine may be the precipitating mechanism.

Proctalgia fugax has been defined as recurring attacks of distressing rectal pain with no local positive findings in the rectum. I have encountered 24 cases which were subjected to extensive study and form the basis of this report.

The ages of the patients varied between 18 and 65 years. This wide variation permitted patients to be observed in the very early stages while they had a vivid recollection of the exact mode of onset, as well as others with forty years' experience who gave a very accurate account of the course of the disease. Patients in the middle age group, on the other hand, gave a more detailed account of symptoms, principally of pain, its precipitating causes, and methods for its relief.

Descriptions of proctalgia can be traced back in the literature to Dr. Myrtle of Harrogate in 1883, and Ewing's (1953) later writings refer to this author. The actual name 'proctalgia fugax' was first used by Thaysen in 1935 (Thaysen, 1936).

INCIDENCE

Several authors join Ewing (1953) in noting the relative infrequency of proctalgia. The relatively high incidence among doctors is also stressed (Ewing, 1953).

Males suffer more commonly, with a maximal age incidence between 20 and 50 years. Many reports suggest that the trouble may first begin in early childhood (Biologist, 1952), and the disease is often familial, the father or mother and several children being affected (C.G.E., 1931; Lynch, 1931; M.C.P.F., 1935).

In my own 24 cases, 15 were men and nine were women. The disease started earlier in the men in this series: their average age of onset was 21 years (limits 18 and 26 respectively) and in women 31 years (limits 26 and 34).

The severity of attacks gradually increased in males to reach a peak at about 26 to 30 years, becoming gradually more spaced and less severe thereafter. Attacks continue in old age but become very mild. In women the disease is more prolonged so that even at 50 years of age some patients continue having severe attacks.

Only two of the patients were doctors, a young general practitioner of 26 and an older physician of 43.

Very significant is the distribution of cases in different kinds of practice as shown by the following facts:

In a general hospital (*Kasr-El-Aini* University Hospital) where the patients are of the poorer working classes only one patient with proctalgia was seen. He was a school teacher who had been operated upon for piles mistakenly supposed to be responsible for the pain. Naturally the proctalgia remained unrelieved. Otherwise I have never met the disease in the poorer working and farmer classes.

The situation was altogether different among Cairo University students presenting for treatment at the Student Hospital:

TABLE I

SURGICAL OUT-PATIENT CONSULTATIONS IN 1958-59

Total number of surgical cases		1,004
Total number of rectal cases		190
Haemorrhoids	107	
Anal fissure	30	
Anal fistula	12	
Pilonidal sinus	14	
Proctalgia fugax	8	
Abscess	5	
Proctitis	2	
Rare conditions: skin tags, polyps, P.O. stricture, etc.	12	

It is thus seen that proctalgia fugax among this class of patients accounts for 4% of all rectal cases or 0·8% of surgical complaints, outranking perianal abscess. These students are given first class medical care and present at the hospital out-patient department for very minor ailments such as boils: it is thus reasonable to assume that at their age group (16 to 26 years) proctalgia is responsible for 4% of all rectal complaints.

All the students were males, and cases cropped up principally in April and early May just before the rigorous annual examinations. Students worried not because of pain, but because of lack of sleep and their fear that they would not be well enough to sit for their examinations. In all these patients rectal examination was negative. The average age was 21 years.

In private practice, of my own 1,024 rectal cases (1949-59) proctalgia was encountered only five times, *i.e.*, an incidence of 0·5%, eight times less commonly than among university students. The average age for all the private rectal cases was 33 years. In contrast to the university students private patients with proctalgia did not seek advice unless they were complaining of an organic or tangible condition in the anus, *e.g.*, bleeding per rectum or second degree piles. Yet there is no reason to believe that proctalgia is less common among these patients than among students. It is possible that students seek advice earlier because they worry that they may be incapacitated at the time of examinations. Especially is this the case since the students are experiencing the attacks for the first time and have as yet not got accustomed to them, nor can they foretell their ultimate course.

It is indeed probable that proctalgia is commoner than one is led to suspect, as was spectacularly demonstrated by my accidentally discovering 10 patients who had never complained. At a party a close friend of my own was suddenly taken ill and retired to an adjacent bedroom, where he proceeded to take off his shoe, fold his leg under him in a chair, and sit with his heel tightly pressed against the anus. He explained this extraordinary behaviour by the fact that since the age of 22 he had been subject to recurring attacks of severe rectal pain, and had been taught to relieve the pain in this manner by his mother who herself suffered from proctalgia. In turn the mother had received the advice from a grandmother, and closer questioning revealed that 10 members of the family suffered from proctalgia. None of these patients had sought medical advice in spite of being wealthy, well educated, and accustomed to seek advice for the most trivial of complaints. Similar families must exist and remain unknown to physicians or statisticians.

MODE OF ONSET

In its commonest form the disease starts with nocturnal attacks of pain. Other ways of onset are less common and the patients ultimately develop the nocturnal attacks. In a typical case (No. 20 of this series) pain is first felt in the rectum late at night or awakens the patient from sleep. The pain is not particularly severe, the patient falls asleep, and next morning the attack is forgotten. A month later a severe attack occurs and is repeated for four or five times. The next severe attack awakens the patient from sleep and this time the severe rectal pain is accompanied by a lower abdominal cramp-like pain. The patient usually feels sick, perspires freely, and syncope is marked. The attack lasts 10 to 15 minutes and ends by the passage of flatus and the patient falling asleep.

Less frequently (two cases) the pain first comes while straining at stool and is repeated at intervals of two to three weeks, then it is succeeded by severe pain on defaecation associated with syncope. The patient may be forced to lie prostrate on the floor until the attack passes. Sooner or later typical nocturnal attacks develop. The patients, however, always remain subject to attacks during defaecation. Pain never follows an easy, massive evacuation, in which the whole left colon participates, but always follows straining to empty the rectum. On occasion typical attacks follow the use of a glycerine suppository.

A third uncommon occurrence (two cases) is an onset associated with diarrhoea. With a recurrence of diarrhoea, even months later, the patient gets a severe prostrating attack of proctalgia, to be followed by typical nocturnal attacks. Such patients have a great fear of diarrhoea because it may be followed in 24 to 48 hours by a diurnal and more than one nocturnal attack.

Patients who start off with typical nocturnal attacks may later develop attacks following defaecation or spontaneously in the daytime. Also people used to sleeping in the afternoon may develop serious attacks during their siesta.

CLINICAL FEATURES

Like epilepsy attacks of proctalgia may be classified into attacks of 'petit mal' and 'grand mal'. The severe attack has a definite aura, while the mild one does not. Typically they occur between 4 and 5 a.m.; on rare occasions more than one attack occurs in the same night. During periods of anxiety or fatigue attacks may occur on several consecutive nights. An average interval of about one month between attacks is common.

The severe attacks of 'grand mal' have an aura which is localized to the lower abdomen and is of a vague nature difficult to describe. The patient becomes aware that the pain will occur about half a minute before the attack, and may wake up from sleep before any pain. The pain itself is deep seated or high in the rectum, severe and agonizing, lasts

10 to 15 minutes and is accompanied by marked syncope. On the rare occasions where rectal examination was possible during a severe attack, I noticed that the sphincters were relaxed or patulous. There was no evidence of spasm in the rectum as far as the finger could reach.

The mild attack 'petit mal', is felt lower down in the rectum, lasts much longer (20 to 90 minutes), is never accompanied by syncope, and clinical examination during the attack showed the sphincters to be spasmodic on several occasions.

The patients are more worried about the syncope than the pain. They keep beside their beds more stimulants than sedatives. Such remedies as brandy, ephedrin, cardiazole-ephedrin, coramine drops, smelling salts, and eau de cologne are found at the bedside of patients more usually than supposedol and spasmocibalgine suppositories, for example.

Some of them are so frightened that they carry medications with them to the lavatory and even keep them in their pockets in case they develop the pain outside their homes.

At the start of the disease attacks are invariably of the petit mal type, but soon pass to the severe type. As the patients get older they once more revert to the milder variety and attacks become more widely spaced. Once in a while an attack of milder character may end as a severe attack. Rarely syncope precedes the pain.

At the height of an attack there is an intense desire to empty the rectum. Attempts to do so are very painful and even if successful fail to relieve the attack. Passage of flatus invariably follows the severe but not the mild attacks.

The association of priapism or erotic dreams is reported by several authors but were not encountered in this series. On a few occasions severe attacks developed in my patients during or at the beginning of coitus. Erection immediately disappeared. Priapism frequently follows severe attacks and sexual desire is intense. Coitus following severe attacks is reported as being very satisfying.

Exciting causes that precipitate attacks are very variable and may be related to work, mental condition, physical or sexual factors. A change of climate has affected several of my patients favourably while they were on vacation. Local inflammation (colitis) in the colon may be a definite exciting factor as may certain foods or drugs that induce loose bowel motions; of special mention in this category are broad-spectrum antibiotics, sulpha drugs, serpasil, shrimps, eggs, and chocolate.

In the diagnosis of proctalgia one should avoid incriminating coexisting local anal lesions, and should not confuse the rectal crises of tables and of anal fissures.

AETIOLOGY

Several authors join Ewing (1953) in postulating a psychoneurotic or nervous origin for the disease. It is a more frequent occurrence in the sophisticated and higher social grade of patients, and the commoner incidence of attacks under conditions of stress, anxiety, or preoccupation, do indeed corroborate this view. Others link the origin with various causes, such as erythromelalgia, scybalous impaction, or even some kind of epilepsy.

I, however, discovered in Bolen's (1943) sigmoidoscopic findings, quoted by Ewing, during an attack of proctalgia a possible clue to the aetiology. Bolen's report is unique in the literature; sigmoidoscopy in a man aged 60 during an attack revealed a reddened, swollen mucosa with prominent vessels. Pressure with the tip of the instrument at the central point of the levator caused mild spasm. Difficulty was encountered in negotiating the recto-sigmoid and gas escaped as soon as the corner had been turned. That the attacks evoke this triad of swollen mucosa, prominent vessels, and obstruction at the recto-sigmoid recalls the episodic vascular attacks involving the superficial temporal artery in cases of migraine. Moreover it may be noted that attacks of migraine are precipitated or aggravated by factors somewhat similar to those that are associated with proctalgia.

In the present series the patient with the peculiar family incidence involving 10 members of his family has already been referred to. One of the sufferers had migraine. It is possible to envisage a train of events explaining severe attacks of proctalgia. To start with vessels at the rectosigmoid junction may suddenly become dilated, to be followed by congestive swelling of the overlying mucosa, which, propelled by the peristaltic wave, may become prolapsed down the rectum for some distance. At this stage sigmoidoscopic findings will correspond to the findings described by Bolen. Localization of primary changes in this area explain both high rectal and lower abdominal or pelvic pains seen in severe attacks. The escape of gas at the end of the attack would follow spontaneous reduction of the oedematous mucosa. Strong support for this hypothesis is lent by the relief that many patients experience on adopting a posture which raises the sigmoid into the abdomen and away from the rectum. Thus many are relieved by adopting the 'genu-pectoral position', and at times attacks may be aborted or serious ones transformed to a mild variety by adopting this posture during the 'aura' phase. Likewise firm pressure directed upwards from the anal region, as previously described, tends to push the anus and rectal contents upwards and bring about relief of pain.

Again mild attacks, which I believe occur lower in the rectum, are accompanied by contraction of the sphincters, as to be expected with any painful stimulus in this area. With the severe attacks mucosal prolapse at the recto-sigmoid induces the intense desire to defaecate with the natural response of relaxed sphincters.

The autonomic nervous system is also undoubtedly involved in proctalgia, as the syncope that accompanies severe attacks is entirely out of proportion to the pain.

TREATMENT

The real difficulty in treating proctalgia lies in preventing attacks. This is practically impossible, and all efforts directed towards this end have failed so far.

Amyl nitrite is reported to bring immediate relief. However it has little or no effect on the 'petit mal' type of crisis. If anything it may be effective in severe cases, attacks of which are in any case, so short lived that relief can hardly in my opinion, be attributed to these drugs.

Gabriel (1948) advises neurotrasentine, or phenobarbitone for highly nervous individuals requiring continuous treatment. Many of our patients are almost neurotrasentine addicts, and many have been taking barbiturates for years with no significant effect on the attacks.

The use of suppositories containing different sedatives and antispasmodics is advocated by some authors. They seem to have little effect. The use of antihistaminic drugs is equally futile. Antimigrainous drugs here not tried because this idea occurred to me only recently, and the drug should prove itself of value against migraine first.

Many patients at the beginning of the attack take stimulants such as coramine, or sedatives such as aspirin or novalgin or both, but the attack is naturally relieved before either has time to act. In mild cases, stimulants are not needed, and sedatives have some effect.

There are many other ways, such as the app'ication of heat to the anus, taking a hot or a cold drink or some food.

In my opinion, the best way to deal with the condition is to reassure the patient of the absence of any serious organic rectal lesion. To bring about as much mental and physical relaxation as possible postural treatment is the most effective. Once the attack is impending, the genu-pectoral position can be adopted, or better still, the patient is advised to lie down on the right side with the buttocks slightly elevated on a pillow, and this should be supplemented by firm pressure on the anus using the left hand for that purpose. This may abort or greatly alleviate a severe attack.

It is evident that the patient should avoid the kind of food or drug that, in his experience, precipitates the pain.

BIBLIOGRAPHY

Abrahams, A. (1935). *Lancet*, **2**, 455.
Aird, I. (1957). A Companion in Surgical Studies, 2nd ed., p. 940. Livingstone, Edinburgh.
Bett, D. H. (1952). *Brit. med. J.*, **1**, 880.
Biologist (1952). *Lancet*, **1**, 52-53.
Blyth, W. (1935). *Ibid*, **2**, 404.
Bull, H. C. H. (1952). *Ibid*, **1**, 424.
Carlill, H. (1935). *Ibid*, **2**, 742-743.
Carter, H. S. (1935). *Ibid*, **2**, 372-373.
'C.G.E.' (1931). *Brit. med. J.*, **2**, 928.
Ewing, M. R. (1953). *Ibid*, **1**, 1083-1085.
El-Mofty, A. (1959). Personal communication.
Gabriel, W. B. (1948). *The Principles and Practice of Rectal Surgery*, 4th ed., p. 166. Lewis, London.
Hughes, T. (1952). *Brit. med. J.*, **1**, 880.
Ibrahim, H. (1960). *Kasr-el-Aini Journal of Surgery*, Vol. I, No. I.
M.C.P.F. (1935). *Lancet*, **2**, 581.
Pybus, F. C. (1946). *Brit. med. J.*, **1**, 824.
'Septuagenarian' (1935). *Lancet*, **2**, 404.
Sheppard, M. D. (1952). *Brit. med. J.*, **1**, 880.
Thaysen, T. E. H. (1936). *Lancet*, **2**, 793-794.
Tidy, H. (1952). *Ibid*, **1**, 314.

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Iron deficiency after partial gastrectomy

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SYNOPSIS Iron-deficiency anaemia will develop in half the males and nearly all females after partial gastrectomy, and in addition to causing the anaemia the sideropenia may cause any general symptoms such as lassitude, failure to maintain weight, or dumping symptoms.

The variable response to treatment is due to the fact that ferrous iron is not adequately released from coated tablets taken after meals and rapidly emptied from the gastric remnant. Treatment with chelated iron is more reliable, and one tablet taken on lying down each night is adequate in the treatment of mild anaemia and for its prevention in women during the reproductive life.

PART I: AETIOLOGY

After partial gastrectomy iron deficiency is common and notoriously difficult to treat orally (Owren, 1952; Goldeck and Gadermann, 1954; Wallensten, 1954). The object of this study was to clarify the relative importance of the many suggested causes with a view to clinical management.

MATERIAL

All patients of this hospital who underwent partial gastrectomy for a benign ulcer in 1954 and 1955 were followed up with the results shown in Table I.

TABLE I

RESULTS OF FOLLOW-UP OF 347 PATIENTS SUBMITTED TO PARTIAL GASTRECTOMY FOR BENIGN ULCERS DURING 1954 AND 1955

Sex	*Male*		*Female*	
Type of operation	*Billroth I*	*Polya*	*Billroth I*	*Polya*
Dead	6	12	1	0
Untraced	17	38	3	4
Did not attend	0	1	1	1
Excluded because of a disease with effects on the haematology	2	2	0	0
Seen in person	56	124	18	26
Seen at local hospital	7	5	3	3
Totals seen	63	129	21	29
		242		

The patients were evenly distributed with regard to age, had had similar pre-operative selection and care throughout, were all within three to five years of operation, and were well documented, thus being suitable subjects for statistical analysis.

METHODS

After an interview to elicit details of post-operative diet (from which the average daily intake of iron in food was calculated, using Scientific Tables, J. R. Geigy, S.A., Basle, 1956) consumption of alkali and aspirin, and any blood loss or treatment, patients were weighed and venepuncture performed.

Haemoglobin was determined (Gibson and Harrison, 1945) in duplicate, reducing the technical error to $\pm 0{\cdot}5\%$. The 6% to 11% variation (McCarthy and Van Slyke, 1939) associated with exercise, meals, menses, etc., was minimized by collecting all blood samples at the same time of day and menstrual cycle from subjects who had rested half an hour or more and whose last meal had been taken at least four hours previously. Preliminary tests had shown these precautions reduced the variation to 2%.

Wallensten (1954) used as normal haemoglobin levels $15{\cdot}4 \pm 0{\cdot}9$ g./100 ml. for males and $13{\cdot}4 \pm 0{\cdot}9$ g./100 ml. for females, so that the criteria for anaemia were levels below 13·6 g. for males and below 11·5 g. for females. Levels before operation or haemorrhage were available in 181 of 192 male patients and agree well apart from the wider scatter of random sampling; $15{\cdot}1 \pm 1{\cdot}3$ g./100 ml. Subsequent experience showed (Fig. 1) that after treatment the haemoglobin levels of females rose to within the range expected for normal males, so that one doubts whether the sex difference is physiological.

Haematocrits were determined on all blood samples, and in general the calculated M.C.H.C. confirmed cases of iron deficiency; however, as 16 patients with a normal M.C.H.C. responded to iron therapy, this index is of limited value as an indicator of the need for treatment with iron.

Serum iron was estimated (King and Wootton, 1956) in duplicate, yielding a technical error of $\pm 5\mu$g./100 ml.

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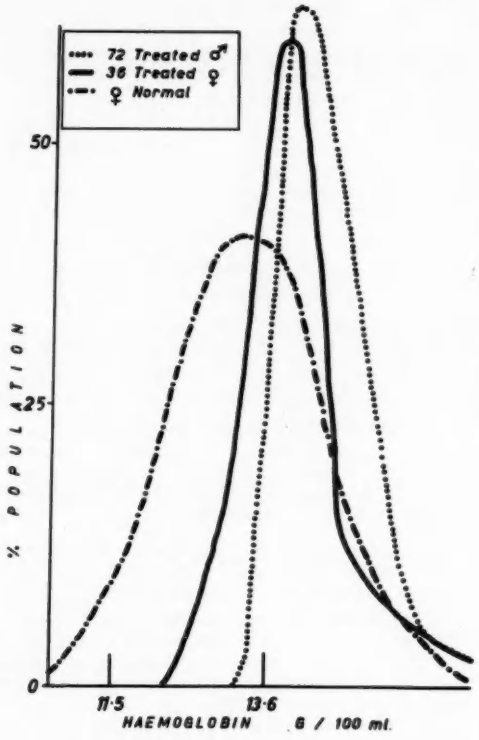


FIG. 1. *Frequency distributions of haemoglobin levels of 'normal' females compared with the treated females and males of this consecutive series, suggesting the 'normal' range is not the physiological.*

In anaemic patients the results generally confirmed the iron deficiency, though 13 anaemic patients with levels greater than 90 μg./100 ml. responded to treatment with iron, revealing that serum iron estimations do not indicate precisely the need for such treatment.

Unsaturated serum iron-binding capacity was measured using radioiron (Feinstein, Bethard, and McCarthy, 1953) with a reproducibility of ± 15 μg./100 ml.

After the exclusion of haemorrhoids, occult blood tests on three specimens of fresh stool from each anaemic patient were made using an o-tolidine tube method matching Gregersen's test, which is recognized to become weakly positive with about 0·5 to 3·0 ml. of blood loss daily (Hughes Jones, 1958). As false positives are obtained from ingested blood, radishes, etc., each of the six positive patients was carefully dieted and 12 successive stools were tested, when five patients became negative. When true occult blood loss is a cause of anaemia it is not usually so difficult to detect.

Serum B_{12} levels were assayed using *L. leichmannii* and were kindly done by Dr. P. Sewell, then at Kingston General Hospital.

Assessment of factors. Males were classed according to the presence or absence of a factor which might be causing anaemia; from the difference in incidence of anaemia in these two groups the contribution of the factor was calculated (Table II) and tested by the χ^2 method. Although no single factor contributed to a statistically significant degree, most are positive so that an iron balance sheet has been compiled to allow assessment of them additively (Table III).

RESULTS AND DISCUSSION

THE CASE FOR TREATMENT WITH IRON ALONE In this series anaemia always responded to treatment with some form of iron and no patient needed any other treatment. (All serum B_{12} levels were within normal limits.)

By Wallensten's criteria about half the males and

TABLE II

ANALYSIS OF POSSIBLE AETIOLOGICAL FACTORS OF ANAEMIA IN MEN AFTER PARTIAL GASTRECTOMY

Factors Studied	*Factor Absent*		*Factor Present*				*Total Anaemic Patients*	*Incidence of Anaemia (%) Possibly due to the Factor*	*χ^2 Test (none significant)*
	No. of Patients	*No. with Anaemia*	*No. of Patients*	*No. with Anaemia*	*Expected if Factor Absent*	*Excess Attributable to the Factor*			
Pre-operative anaemia	128	47	65	34	24	10	81	12	6·7
Anaemia induced by operation	55	18	63	22	21	1	40	3	0·6
Iron losses: Post-operative: overt	158	59	49	22	19	3	81	4	2·8
Iron losses: occult	207	83	1	1	nil	1	84	1	—
Iron intake in diet (10 mg./day)	116	38	65	27	21	6	65	9	3·7
Iron absorption reduced by taking alkali (no blood loss)	175	68	29	16	11	5	84	6	—
Iron transport, serum total iron-binding capacity	177 Mean = 292 μg./100 ml. σ = 54 μg./100 ml.								
Aspirin consumption	141	55	64	29	25	4	84	5	2·8
Serum B^{12} level	99 Mean = 435 μμg./ml. σ = 183 μμg./ml.								
Time since operation, four years	59	19	108	37	35	2	56	—	—
Age greater than 50 years	77	33	133	53	57	—4	86	—	—
Type of ulcer, gastric	165	67	42	19	17	2	86	—	—

TABLE III

ANAEMIA CAUSED IN 84 MALES AFTER PARTIAL GASTRECTOMY

		% from Table III
Abnormal iron losses	Pre-operative	12
	Per-operative	3
	Post-operative	5
Abnormal iron intake	Less than 10 mg./day	9
	Aspirin consumption	5
	Total	34

half the females were anaemic, but if the same standards are applied to females as to males, nearly all the females were anaemic (Table IV). After treatment, the haemoglobin levels of both males and females fell within the normal male range except for one man (13·3 g.) and three women (12·6, 12·8, 13·3 g.). The view that iron deficiency was clinically significant is supported by the observation that even when the haemoglobin rise was small, the patient often felt markedly better and 24 of 108 gained weight for the first time since operation. Of the non-anaemic males 23% had serum iron levels below 60 μg./100 ml. which was considered to constitute sideropenia (Waldenström, 1946), and the few of these who were treated responded subjectively in the same way as anaemic patients and gained weight. These observations confirm those of Reimann (1956) and Beutler, Larsh, and Gurney (1959) who have shown that sideropenia has a profound effect on energy, muscle mass, and sexual libido, and support those of Ott and Jasinski (1954) who often cured patients of their dumping symptoms by treating the sideropenia which was invariably present.

TABLE IV

INCIDENCE OF ANAEMIA AFTER PARTIAL GASTRECTOMY[1]

	Present Series Alone			*Present series and series of Watson (1947), Lyngar (1950), and Wallensten (1954)*	
	No. of Patients	*No. with Criterion*	*% Anaemia*	*No. of Patients*	*% Anaemia*
Females	50	Hb<11·5g. 21	42	227	40
		Hb<13·6g.			
Males	208	84	40	715	49
Females	50	48	96	207	94

[1]This series includes five patients treated for anaemia who are assumed to have had a haemoglobin level below 13·6 g./100 ml.

THE PATHOGENESIS OF THE IRON DEFICIENCY Iron deficiency in all these cases must be due to present or past blood loss, or to an inadequate supply of iron. Women who have not passed the menopause lose 500 to 3,000 ml. of blood a year, and of 23 such patients all had a haemoglobin level below 13·6 g., which was exceeded in all cases after treatment. Because of this apparently dominant effect of menstruation, other factors were analysed only in male patients.

Before transfusion became freely available (Morley and Roberts, 1928), survivors from partial gastrectomy for haematemesis were grossly anaemic. Nowadays pre-operative iron loss is less important (Table II) and its contribution can be abolished by adequate transfusion and treatment with iron during convalescence. These same measures minimized the subsequent effects of pre-operative loss, which initially rendered over half the patients anaemic. In the years following operation, overt blood loss, chiefly from haemorrhoids, was of little general significance and individual cases are treated on their own merits. Occult blood loss is rare (Table V) and the speculations of Smith and Mallett (1957) are without foundation. There is good evidence against the hypothesis of intermittent loss in that only eight weakly positive occult blood reactions were found in 321 random stools, corresponding to a loss from the average patient of 24 ml. of blood in 321 days, whereas the physiological loss over this period is about 90 ml. Three cases of acute melaena have been ascribed to aspirin consumption, but if these are excluded only 5% of the anaemia could have been caused by aspirin. A low dietary iron intake, possibly causing 9% of the anaemia, was usually due to the eating of small meals as an adaptation to the symptoms of dumping: this constitutes a vicious circle as the dumping symptoms are aggravated by iron deficiency. The age of the patient and the site of the ulcer appear irrelevant.

TABLE V

OCCULT BLOOD LOSS AFTER PARTIAL GASTRECTOMY

Series	*No. of Patients Examined*	*No. of Patients Found to be Positive*
Dedichen (1934)	47	1
Hartfall (1934)	20	1
Lyngar (1950)	146	Rarely
Blake and Rechnitizer (1953)	104	4
Baird and Wilson (1959)		
Not anaemic	32	6
Anaemic	30	3
Choudhury and Williams (1959)	40	0
Stevens *et al.* (1959) Over 1 year	5	0
This series	84	1

The factors considered above account for only one third of the anaemia so that the only factor not yet considered, that is, defective iron absorption, must account for the remaining two thirds.

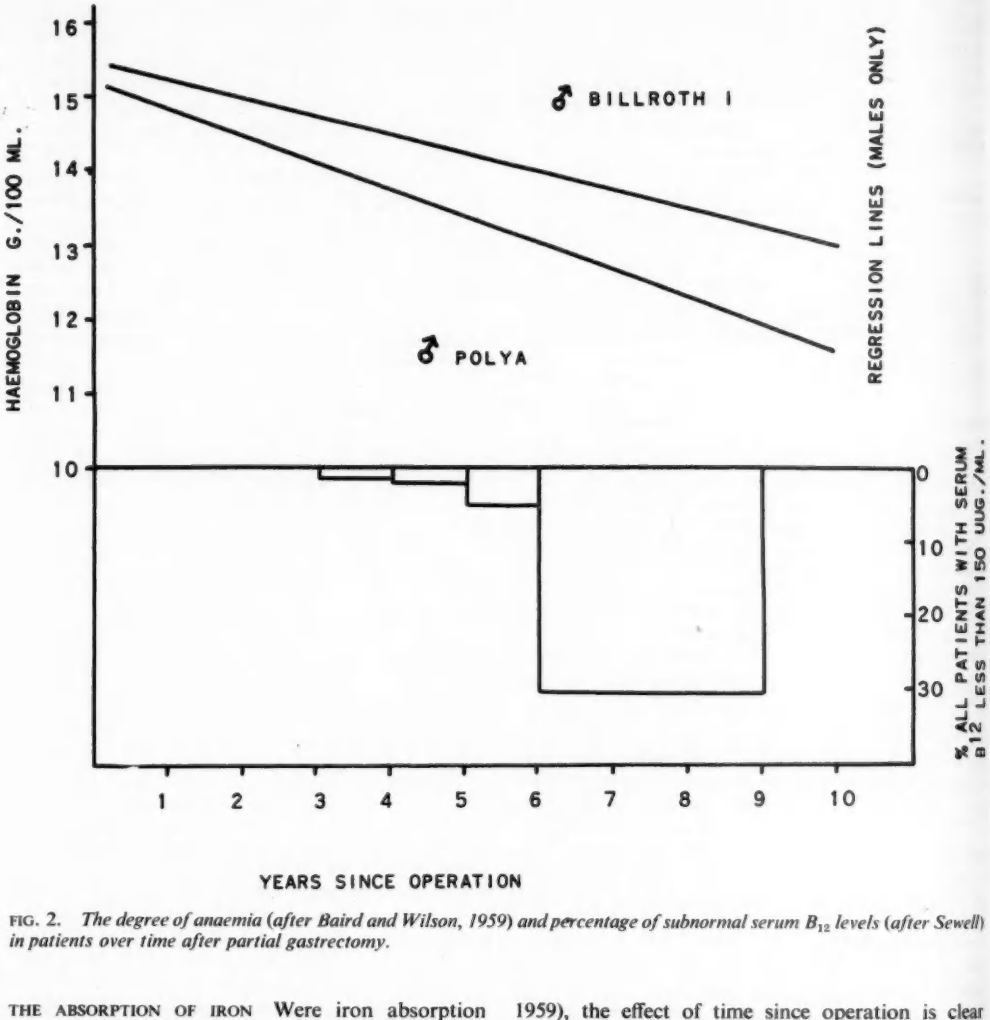


FIG. 2. *The degree of anaemia (after Baird and Wilson, 1959) and percentage of subnormal serum B_{12} levels (after Sewell) in patients over time after partial gastrectomy.*

THE ABSORPTION OF IRON Were iron absorption suddenly to cease, a normal man with 1,000 mg. of iron in store and 2,500 mg. in haemoglobin (15 g./100 ml.) would live for three and a half years before his haemoglobin fell below 13·6 g./100 ml., assuming the reasonable loss of 1 mg. of iron daily (Moore, 1955). As most patients were seen more than three and a half years after operation, the criterion of 13·6 g. used in this series and Wallensten's fails to detect an increasing incidence of anaemia with the later years. However, by using a criterion of 12·6 g. (Burge, 1960), or better still, by plotting haemoglobin levels directly against time (Baird and Wilson, 1959), the effect of time since operation is clear (Fig. 2). Sewell (data to be published) has shown that serum B_{12} levels behave similarly (Fig. 2), and their correlation with the iron deficiency suggests that 'sideropenic gastritis' may impair the absorption of B_{12}: since the achylia is initially reversible (Brumfitt, 1960). This hypothesis could explain the spontaneous rise of serum B_{12} levels after iron therapy alone (Cox, Meynell, Gaddie, and Cooke, 1959).

If ferrous ions are presented to any level of the small gut of the cat (Jacobi, Pfleger, and Rummel, 1956) or of man (Holmberg, Vahlquist, and

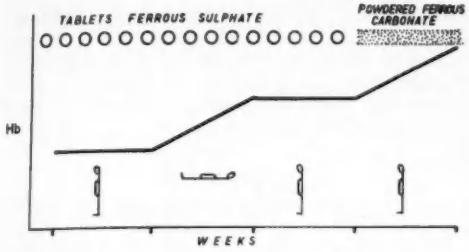


FIG. 3. *The influence of posture and disintegration of tablets on iron absorption after partial gastrectomy (after Owren, 1952).*

Waldenström, 1944) they are absorbed. In healthy individuals the pancreatic secretion renders the contents of the duodenum neutral or slightly alkaline, so lowering the concentration of ferrous ions that iron is mainly absorbed in the duodenum. It is known that the Polya gastrectomy is followed by more severe anaemia than the Billroth I operation, and Wallensten suggests that this is due to exclusion of the duodenum. Choudhury and Williams (1959) confirmed this difference, but found that patients with gastroenterostomy, in whom the duodenum was no longer a pathway for digesting food, absorbed iron normally. Anaemia only seems to occur when the distal part of the stomach is resected (or excluded, Lublin, 1936), and this area is known to regulate the rate of emptying of the stomach (Thomas, 1957). An alternative hypothesis thus seems to be that failure of absorption is due more to the rapid passage of iron through the upper gut; not only is there less time for ferrous ions to be split off from food (or therapeutic tablets) but they are shorter lived. Radiologically, hurried emptying of the gastric remnant was recorded by Hartfall in 1934, and in 1952 Owren (confirmed by Goldeck and Gadermann, 1954) showed that in the erect posture tablets might reach the terminal ileum within 20 min. and that these patients would only respond when confined to bed, as lying down slowed the transit of the ferrous sulphate tablets so that they disintegrated in the stomach (Fig. 3). Powdered ferrous carbonate in large doses could be effective while patients are ambulant, but Owren admits it is difficult to persuade patients to take this.

These authors alone, among the many who have studied iron absorption after partial gastrectomy (Table VI), have considered the influence of posture and of the physical form in which iron is presented. If ferrous iron in solution is administered to fasting subjects (especially in bed), maximum absorption must occur, yet the presence of food can halve such absorption (Bothwell, Pirzio-Biroli, and Finch, 1958). Consequently such studies reproduce neither the usual conditions of clinical treatment (tablets after meals) nor the natural dietary background against which the iron deficiency develops.

Malabsorption of iron in food has, in fact, been demonstrated after partial gastrectomy (Table VI), and alkali consumption (Table II); achylia *per se* (Bothwell *et al.*, 1958), jejunitis, and steatorrhoea are of little significance in its causation.

Unsaturated serum iron-binding capacity may be of importance in iron absorption (Hallberg, 1960), and in iron-deficiency anaemia levels above normal (over 300 μg./100 ml.) are found in association with the known increased ability to absorb iron. The

TABLE VI

RESULTS OF STUDIES OR IRON ABSORPTION AFTER PARTIAL GASTRECTOMY

Authors	*Method*	*No. of Patients*	*Evidence for Malabsorption*
Using inorganic iron in fasting patients			
Owren (1952)	Radiological	2	Yes
Goldeck and Gadermann (1954)	Radiological	3	Yes
Wallensten (1954)	Plasma iron absorption curves, which can be misleading (Bothwell *et al.*, 1955)	4	Yes
Heinrich (1954)	Plasma iron absorption curves, which can be misleading (Bothwell *et al.*, 1955)	7	Yes
Murray *et al.* (1957)	Plasma iron absorption curves, which can be misleading (Bothwell *et al.*, 1955)	2	Yes
Zingg *et al.* (1959)	Plasma iron absorption curves, which can be misleading (Bothwell *et al.*, 1955)	83	No
Smith and Mallett (1957)	Radioiron balance	43	No
Baird and Wilson (1959)	Radioiron balance	13	No
Duthie (1959)	Radioiron balance	4	No
Using inorganic iron added to food			
Bothwell *et al.* (1958)	Double isotope balance	5	Yes
Stevens *et al.* (1959)	Double isotope balance	8	Yes
Choudhury and Williams (1959)	Radioiron balance	25	Yes
Using radioiron incorporated in food			
Chodos *et al.* (1957)	Radioiron balance	1	Yes
Baird and Wilson (1959)	Radioiron balance	4	Yes

latter could not be demonstrated after partial gastrectomy (Baird and Wilson, 1959), and in 84 anaemic patients no such increase in unsaturated serum iron-binding capacity could be found (225 ± 64 μg./100 ml.): however these results probably reflect the insidious onset of the chronic anaemia, for the described increases (Laurell, 1947) were found in acute cases.

After consideration of the many factors affecting iron absorption after partial gastrectomy it can be said that the most significant cause of the observed malabsorption is an increased rate of emptying of the gastric remnant.

SUMMARY

An analysis of the literature and a study of 242 patients reveals that, after partial gastrectomy, iron deficiency (1) leads to anaemia in half the males and, because of menstruation, in nearly all the females; (2) apart from causing anaemia, can have far-reaching effects on the other tissues of the body; (3) insidiously develops over the years as a result of the malabsorption of iron from food due chiefly to the rapid emptying of the gastric remnant.

This study has enabled the following principles of management to be defined: (1) Haemoglobin levels should be determined every three months in menstruating women and yearly in other patients. (2) Iron therapy should be given to all patients with a haemoglobin level below 13·6 g./100 ml.; continuously, as prophylaxis, to menstruating women; and is worth a trial if sideropenia without anaemia is suspected; in patients complaining of lassitude, failure to maintain weight, or dumping symptoms.

PART II: TREATMENT

PRINCIPLES

The case for iron therapy has been stated in Part I of this paper: and parenteral treatment should only be used when there is no alternative.

The object of oral therapy is to present soluble iron to the small gut. Solutions of ferrous salts are unpalatable, are rapidly rendered insoluble by the alkaline contents of the upper small intestine, and are unstable, being readily oxidized to the ferric state with its obnoxious staining and side-effects. They need to be freshly prepared as an elixir and this is expensive and impractical. Tablets, although convenient, would require very rapid dispersal unless taken lying down. Disintegration times *in vitro* can be misleading as to what actually happens *in vivo* (Weiner, 1960) but it will be shown that they are of value in iron therapy. Tablets harden with age (Table VII) and a small survey of current iron preparations from the shelves of local pharmacies revealed that, as judged by disintegration times[1] (Table VII), many of the tablets would be unsuitable for patients after gastrectomy.

TABLE VII

CONDITION OF IRON TABLETS BEFORE REACHING PATIENTS

		Disintegration Time (min.)
Ferrous sulphate tablets of a reputable firm		
Time since manufacture	Fresh	20
	One month	30
	Three months	40
	Six months	60
	Twelve months	85
Tablets of various ferrous salts from shelves of local pharmacies		
	Range	20-140
	Average	40-80

Chelated iron preparations consist of iron combined to an organic complex by NH_2 groups in such a way that they are stable in alkaline solutions (Arden, 1950) and are thus available for absorption along the entire small gut. It has been suggested that they by-pass the ferritin mechanism for very high blood levels were achieved rapidly in cats (Jacobi *et al.*, 1956). The ferritin mechanism itself seems to operate by chelating ferrous ions from the gut, so that chelated iron can be loosely regarded as 'predigested'. Of the preparations then available, ferrous glycine sulphate was chosen, being the simplest chelate and also the cheapest. It is not stable in acid solutions, the ferrous moiety becoming ionic at about *p*H 1·5, so that in stomachs with normal acidity it has no advantage over ordinary ferrous salts. However, after partial gastrectomy, it seemed to offer an ideal treatment.

METHODS

Each patient was given one of four régimes (Table IX) for one month, and then, if the response was bad, the treatment was changed to one of the remaining three and continued until the haemoglobin had risen above 13·6 g./100 ml. (Fig. 4). Only those patients who claimed to have taken over 90% of the prescribed doses are included in the 214 treatments reported. The criteria of response are arbitrary, taking into account the initial haemoglobin level (Table VIII). The monthly gains may seem small, being about half of what would be expected in patients with intact stomachs, but after partial gastrectomy even parenteral therapy results in a slow response; probably because the 7·5 g. of globin, required for each 1% (0·15g.) rise, have to come from a protein balance that is critical (Pattarin, 1958).

[1]Disintegration times were determined by Messrs. Coates & Cooper Ltd., using the rotating gauze method of the British Pharmacopeia.

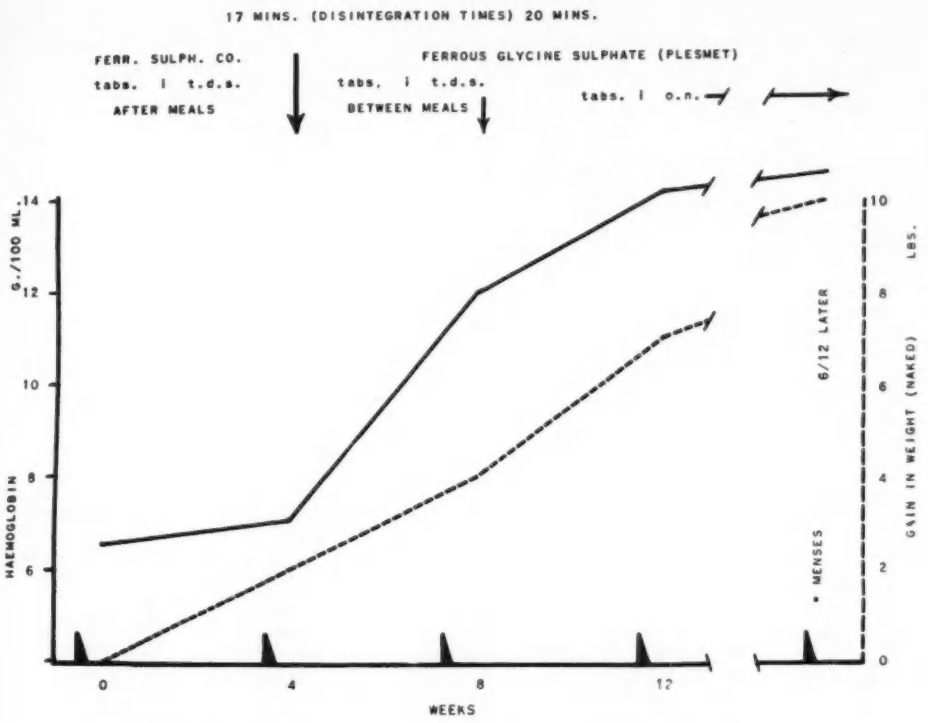


FIG. 4. *The treatment of a female patient after the failure of a 6/52 course of ferrous gluconate tablets (ii t.d.s.p.c.). Though all the tablets rapidly disintegrate* in vitro, *the Plesmet régime was the better and provided adequate maintenance over six months. The weight gain was independent of the haemoglobin response.*

TABLE VIII

CRITERIA OF RESPONSE TO ORAL TREATMENT OF IRON-DEFICIENCY ANAEMIA AFTER PARTIAL GASTRECTOMY

Initial HB (g./100 ml.)	*<9·5*	*9·5-11·5*	*>11·5*
	Rise per Month		
None	—	—	—
Unsatisfactory	<1·05	<0·90	>0·60
Satisfactory	1·05-2·05	0·90-1·65	0·60-0·90
Excellent	>2·05	>1·65	>0·90

RESULTS

The first régime, used routinely in this hospital in the treatment of iron deficiency, is satisfactory for patients with intact stomachs. Table IX shows that after Polya gastrectomy this régime is virtually useless. Even after the Billroth I operation it is not very efficient.

Using treatment, identical except for a more rapid dispersal of the iron tablets, the results after Polya gastrectomy approach those after Billroth I gastrectomy. This supports the hypothesis (Part I) that the rate of emptying of the gastric remnant is more important than duodenal exclusion in the causation of iron malabsorption.

This régime is still inefficient in half the included patients, and as it could not be tolerated by an excluded 20% of patients, it was not tried between meals.

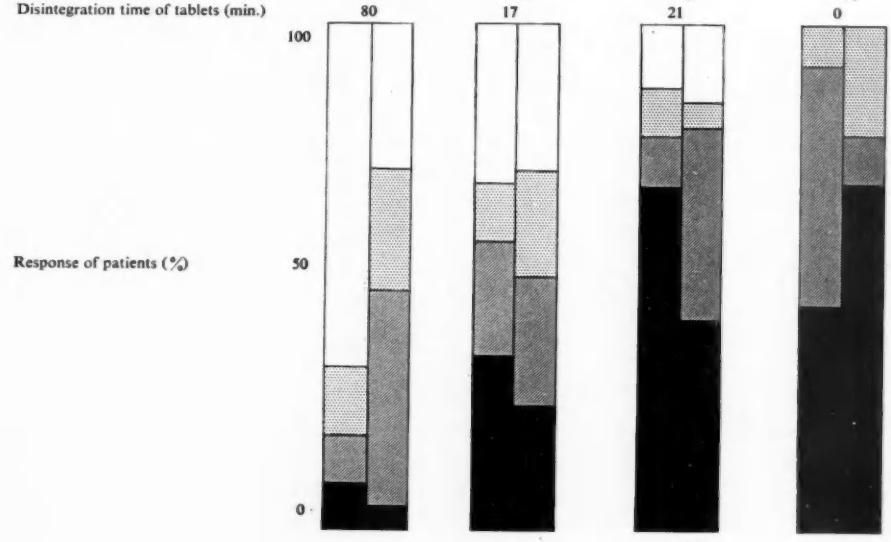
Ferrous glycine sulphate tablets taken between meals yield excellent results, the more remarkable in that all patients to whom it was given had already failed to respond to ferrous sulphate tablets: further, patients with haemoglobins of 11·5 to 13·6 g./100 ml. had taken only one tablet (50 mg. Fe) daily, on lying down at night.

The chelated iron, given as a stable syrup, was

TABLE IX

RESULTS OF ORAL IRON THERAPY AFTER PARTIAL GASTRECTOMY

Regime	*1*	*2*	*3*	*4*
Daily mg. iron	*215*	*215*	*50 or 150*	*50*
	Ferrous Sulphate		*Ferrous Glycine Sulphate*	
	Co. N.F. tabs i with ascorbic acid 100 mg. three times daily After meals		*Tabs iii/day or tabs i nocte Between meals*	*The syrup 1 teaspoonful twice daily*
Rate of intolerance	8%	20%	0%	0%
Disintegration time of tablets (min.)	80	17	21	0



Type of operation	Polya	Billroth I	Polya	Billroth I	Polya	Billroth I	Polya	Billroth I
None	21	6	14	6	6	2	0	0
Unsatisfactory	4	5	5	5	5	1	2	2
Satisfactory	3	9	10	5	5	4	14	1
Excellent	2	1	14	5	33	5	13	7
Number of patients	30	21	43	21	49	12	29	10

immediately available for absorption. This was satisfactory in the three Billroth I and 11 Polya patients who had failed to respond adequately to all tablets taken by day, when presumably 'hurry' had prevented adequate absorption.

The four patients with unsatisfactory response to the syrup all had haemoglobin levels below 11·5 g./100 ml. Their treatment was changed to ferrous glycine sulphate tablets, one b.d. between meals and one taken on lying down at night. At this higher dosage of 150 mg. of iron, they all responded excellently.

DISCUSSION

When tablets, all with a disintegration time of 30 minutes, are given to ordinary patients in a double-blind trial, no difference in efficacy is

detected between many of the current ferrous salts, and a genuine intolerance can be shown in 8% of patients, quite apart from pyschological factors (Berlin, 1960). This coincides with that found in régime 1; thus the 20% rejection of more rapidly distintegrating (17 minutes) ferrous sulphate may be considered as genuine intolerance.

Any attempt to speed up the release of inorganic iron, in an effort to improve absorption, is likely to be rejected by many patients. On the other hand, ferrous glycine sulphate causes minimal side-effects, a finding in agreement with Jennison (1958) and Barnes (1960), presumably because chelation prevents oxidation of the ferrous moiety to obnoxious ferric ions.

It is likely that any tablet, releasing its iron within 30 minutes, will give a satisfactory response if taken lying down. Here, this has only been demonstrated with ferrous glycine sulphate, and one tablet taken last thing every night has been found during the past year to be convenient and adequate in the prevention of a recurrence of anaemia in our menstruating women.

Finally, only the consecutive (unselected) cases from the survey (Part I) are reported here. Experience with some hundred other cases of anaemia after partial gastrectomy supports the findings.

SUMMARY

The notorious difficulties encountered in the oral treatment of iron deficiency after partial gastrectomy are probably due to an inadequate release of ferrous ions from coated tablets, taken after meals and rapidly emptied from the gastric remnant.

Treatment with tablets of the iron chelate, ferrous glycine sulphate, taken between meals is reliable, though a few patients respond better to the stable syrup.

One tablet, taken on lying down each night, is adequate in the treatment of mild anaemia (haemoglobin 11·5 to 13·6 g./100 ml.) and its prophylaxis in women so long as menstruation continues.

I wish to thank Dr. G. Discombe for his advice and encouragement, Dr. F. Avery Jones and the Department of Gastroenterology for access to their patients, records, and laboratories, Messrs. Coates & Cooper Ltd. for a supply of ferrous glycine sulphate (Plesmet), our artist and photographic department for help with the diagrams, and those physicians at other hospitals who kindly saw patients who had moved away from this hospital.

REFERENCES

Arden, T. V. (1950). *J. chem. Soc.*, pp. 882-885.
Baird, I. M., and Wilson, G. M. (1959). *Quart. J. Med.*, **28**, 35-41.
Barnes, R. D. S. (1960). *Practitioner*, **184**, 789-792.
Berlin, S-O. (1960)[1].
Beutler, E., Larsh, S., and Gurney, C. W. (1959). *J. Lab. clin. Med.*, **54**, 791-792.
Blake, J., and Rechnitizer, P. A. (1953). *Quart. J. Med.*, **22**, 419-437.
Bothwell, T. H., Mallett, B., Oliver, R., and Smith, M. D. (1955). *Brit. J. Haemat.*, **1**, 352-357.
——, Pirzio-Biroli, G., and Finch, C. A. (1958). *J. Lab. clin. Med.*, **51**, 24-36.
Brumfitt, W. (1960). *Quart. J. Med.*, **29**, 1-18.
Burge, H. W. (1960). *Postgrad. med. J.*, **36**, 2-12.
Chodos, R. B., Ross, J. F., Apt, L., Pollycove, M., and Halkett, J. A. E. (1957). *J. clin. Invest.*, **36**, 314-326.
Choudhury, M. R., and Williams, J. (1959). *Clin. Sci.*, **18**, 527-532.
Cox, E. V., Meynell, M. J., Gaddie, R., and Cooke, W. T. (1959). *Lancet*, **2**, 998-1001.
Dedichen, J. (1934). *Acta. chir. scand.*, **75**, 242-257.
Duthie, H. L. (1959). *Scot. med. J.*, **4**, 523-526.
Feinstein, A. R., Bethard, W. F., and McCarthy, J. D. (1953). *J. Lab. clin. Med.*, **42**, 907-914.
Gibson, Q. H., and Harrison, D. C. (1945). *Biochem. J.*, **39**, 490-497.
Goldeck, H., and Gadermann, E. (1954). *Ärztl. Wschr.*, **9**, 39-40.
Hallberg, L. (1960)[1].
Hartfall, S. J. (1934). *Brit. med. J.*, **1**, 136-141.
Heinrich, G. (1954). *Ärztl. Wschr.*, **9**, 609-611.
Holmberg, C. G., Vahlquist, B., and Waldenström, J. (1944). *Om järn och järnterapi*. Malmö.
Jacobi, H., Pfleger, K., and Rummel, W. (1956). *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **229**, 198-206.
Jennison, R. F. (1958). *Practitioner*, **181**, 731-735.
Jones, N. C. Hughes (1958). *Brit. med. J.*, **1**, 493-496.
King, E. J., and Wootton, I. D. P. (1956). *Microanalysis in Medical Biochemistry*, 3rd ed., p. 108. Churchill, London.
Laurell, C. B. (1947). *Acta. physiol. scand.*, **14**, Suppl. 46. p. 82.
Lublin, H. (1936). *Amer. J. dig. Dis.*, **3**, 8-10.
Lyngar, E. (1950). *Acta med. scand.*, Suppl. 247.
McCarthy, E. F., and Van Slyke, D. D. (1939). *J. biol. Chem.*, **128**, 567-572.
Moore, C. V. (1955). *Amer. J. clin. Nutr.*, **3**, 3-10.
Morley, J., and Roberts, W. M. (1928). *Brit. J. Surg.*, **16**, 239-252.
Murray, D. H. Jr., Najarian, J. S., Buster, C. D., Scott, K. G., Harper, H. A., and McCorkle, H. J. (1957). *Surg. Forum.*, **8**, 211-214.
Ott, W., and Jasinski, B. (1954). *Gastroenterologia (Basel)*, **82**, 14-19.
Owren, P. A. (1952). *Acta chir. scand.*, **104**, 206-214.
Pattarin, L. (1958). *Arch. ital. Mal. App. dig.*, **24**, 187-197.
Reimann, F. (1956). Demonstration at the International Society of Haematology, Boston. (Personal photostats.)
Sewell, P. Personal communication, to be published.
Smith, M. D., and Mallett, B. (1957). *Clin. Sci.*, **16**, 23-34.
Stevens, A. R., Pirzio-Biroli, G., Harkins, H. N., Nyhus, L. M., and Finch, C. A. (1959). *Ann. Surg.*, **149**, 534-538.
Thomas, J. E. (1957). *Physiol Rev.*, **37**, 453-474.
Waldenström, J. (1946). *Acta med. scand.*, Suppl. 170, pp. 252-279.
Wallensten, S. (1954). *Acta. chir. scand.*, Suppl. 191.
Watson, A. B. (1947). *Brit. J. Surg.*, **34**, 353-366.
Weiner, M. (1960)[1].
Zingg, W., Green, P. T., Thomas, E. J., and Hamilton, V. (1959). *Gastroenterology*, **36**, 806-811.

[1]To be published in 'The Proceedings of the Seventh European Congress of Haematology'. London, 1959. Karger, Basle (1960).

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Some metabolic and haematological effects of oesophago-jejunostomy with by-pass of the stomach

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SYNOPSIS Twelve subjects have been studied in whom oesophago-jejunostomy had been performed leaving the stomach *in situ*. All had considerable metabolic disturbance with steatorrhoea and inability to absorb vitamin B_{12}, and the operation has been abandoned in favour of oesophago-jejuno-gastrostomy.

The need for a radical total gastrectomy for carcinoma of the upper part of the stomach led to the use of the Roux loop of jejunum for restoring intestinal continuity. The nature of the disease, and the pressing indication for removal, forced the hand of the surgeon to leave his patient in what would appear to be a most undesirable physiological state, where the food passed straight from the oesophagus into the small intestine, meeting the bile and pancreatic juice 10 or 12 in. below the anastomosis where a T junction was made between the loop and the duodeno-jejunal flexure (Fig. 1a).

Many of the patients with total gastrectomy led a surprisingly normal life, eating well, though not gaining weight, having a single bowel action a day, and earning their living. If they lived long enough, however, they all developed a megaloblastic anaemia unless they were treated prophylactically with vitamin B_{12}.

The success of oesophago-jejunostomy in these patients led to the adoption of a similar operation for people with severe peptic stricture of the oesophagus. In them, however, there was no necessity to remove the stomach. The need was to remove the stricture and to prevent the digestive juices of the stomach from reaching the oesophageal mucosa which was ill prepared to withstand its corrosive action. The patients were mainly adults and in the same age group as those suffering from carcinoma. It seemed reasonable, therefore, to excise the oesophageal stricture, to close the cardiac end of the stomach completely and to join the oesophagus to a Roux loop of jejunum. In this way the food passed from oesophagus to jejunum without ever entering the stomach. The gastric juice could not enter the oesophagus, and whatever might be secreted would join the bile and pancreatic juice to enter the jejunum and mix with the food at the T anastomosis (Fig. 1b). When the stomach thus became a rather distant cul-de-sac of the alimentary canal it was not possible to determine whether it did in fact continue to secrete normally. This operation was only performed on middle-aged and elderly adults and was never applied to the problem of peptic stricture in young children. It was the use of an isolated loop of jejunum to replace the resected stricture in children (Fig. 1c) that led to the abandoning of the operation described above for adults; but in the meantime a small series of patients had become available for metabolic study which was unique and unlikely to occur again.

In all the operation was performed 40 times. Six of the patients succumbed within six months, two of them from uncontrollable pulmonary tuberculosis. Thirty-four remaining patients gave a general clinical post-operative picture which varied with the condition before operation. Some who were emaciated from prolonged starvation gained weight rapidly, one man increasing from 84 lb. to 154 lb. in about a year, subsequently falling to a steady 127 lb. None ever regained the weight that had been normal before oesophageal stenosis occurred. The men made an outstandingly better metabolic recovery than the women. All had looseness of the bowels, particularly after meals, for periods varying from one to six months, and while many returned to a single formed stool a day, most were subject to attacks lasting one to three days in which they would have three to five

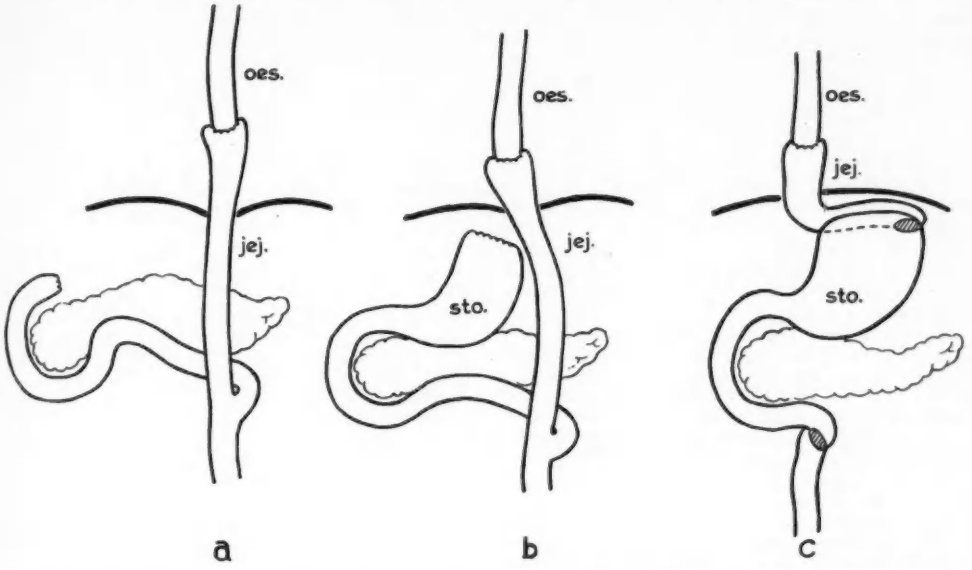

FIG. 1. a *oesophago-jejunostomy with total gastrectomy*, b *oesophago-jejunostomy with by-pass of the stomach*, c *oesophago-jejuno-gastrostomy*.

bowel actions. Practically all had a tendency to feel faint with an increased pulse rate after meals. This was most marked and lasted longer in the women than in the men. This symptom became less with time and was diminished by eating small dry meals and drinking between meals. After a short time some of the men declared that they ate normal meals with the rest of the family and had no discomfort whatever; many of the women continued to rest for half an hour after meals.

One of the most interesting post-operative features was the development of anaemia in several of the subjects. It was primarily to investigate this aspect of the condition that the present study was undertaken.

MATERIAL

All the surviving patients were asked if they would come into hospital for a period for investigation. They had all been operated on in Leeds and this meant their coming to Oxford. Twelve agreed to come. There was a natural tendency for those with troublesome post-operative symptoms to be prepared to come; there was also a preponderance of women in the sample.

This group of 12 who had been subjected to the operation of oesophago-jejunostomy with by-pass of the stomach was compared with two patients who had had an oesophago-jejuno-gastrostomy. Three of the 12 patients were sufficiently disabled to agree to having the oesophago-jejunostomy converted to an oesophago-jejuno-gastrostomy and further observations were made on these three (A.B., E.D., and G.W.) after re-operation.

METHODS

As many as possible of the following investigations were carried out on each subject:—

1 A venous blood sample was taken and the blood count, prothrombin, serum iron, blood urea, serum calcium, phosphate and phosphatase, and the total and albumin fractions of the plasma proteins were estimated. Blood films and sternal marrow were examined.

2 SERUM VITAMIN B_{12} Serum vitamin B_{12} was estimated by microbiological assay with *Lactobacillus leichmannii* as the test organism (Spray, 1955). Using this method Spray found that the majority of patients with pernicious anaemia have values below 100 $\mu\mu$g./ml.; the mean in 92 such patients was 64 $\mu\mu$g./ml. In control subjects values obtained lay between 150 and 1,000 $\mu\mu$g./ml. with a mean of 450 $\mu\mu$g./ml. (Spray and Witts, 1958).

3 THE ABSORPTION OF VITAMIN B_{12} Vitamin B_{12} labelled with radioactive cobalt was used to study absorption of the vitamin. A dose of 0·5 μg. was given to the fasting

subject in about 30 ml. of water. Nothing more was given to eat or drink for a further two hours. The subjects were provided with waxed cardboard containers in which to collect all stools which were passed subsequently and the collections were continued until less than 1% of the dose given was found in two successive specimens. Radioactivity was measured in a double ring of 32 Geiger-Müller tubes (Bradley, 1957). It was assumed that all the activity recovered in the faeces represented the proportion of the dose which was not absorbed and from this the percentage absorption of the labelled vitamin was calculated. In all those who showed abnormal absorption of vitamin B_{12} the test was repeated with the addition of 50 mg. of an active preparation of intrinsic factor (Lederle, 45774-133).

Using this faecal technique we have found that control subjects excrete 14 to 45% of the oral dose (mean 31%) and patients with pernicious anaemia 64 to 100% (mean 88%) (Callender, Turnbull, and Wakisaka, 1954; Retief, 1959), *i.e.*, control subjects absorb 55 to 86% of the 0·5-μg. dose and patients with pernicious anaemia 0 to 36%. The addition of 50 mg. of Lederle 45774-133 preparation of intrinsic factor reduced the excretion in patients with pernicious anaemia to 32 to 57% (mean 47%) (unpublished observations).

In some subjects a further test of absorption of vitamin B_{12} was made, 0·25 mg. of carbachol being given half an hour before the labelled vitamin. In others 0·04 mg. per kg. body weight of histamine acid phosphate was given subcutaneously half an hour after an intramuscular injection of mepyramine maleate (Anthisan) and the dose of labelled vitamin was given half an hour after the histamine injection. With both these procedures it could be expected that there would be an outpouring of gastric secretion which would reach a maximum about 15 minutes after the carbachol or histamine and be maintained for about 30 minutes.

In interpreting the effect of various factors on absorption of labelled vitamin B_{12} the variability of the test should be considered. In 10 reliable subjects who carried out duplicate tests within a short interval of time the results varied by 0 to 9% of the oral dose (mean 4%, S.D. 3%). Taking the 94 subjects who have had duplicate tests, under apparently similar conditions, since the introduction of the test in our department, the difference was as much as 0 to 22% (mean 7%, S.D. 6%) (Deller, Germor, and Witts, 1961). The latter figures include patients who had tests at intervals of several months or even years and may include some who were unreliable in collecting stools although they all denied this source of error.

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7 FAECAL FAT This was estimated by the method of van de Kamer, ten Bokkel Huinink, and Weyers (1949) on four-day collections of faeces with a dietary intake of approximately 70 g. of fat daily. The upper limit of normal was taken as 6 g. per day.

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Age	Sex	Years since Operation	Treatment	Hb (g./100 ml.)	Marrow	Serum B_{12} (µµg./ml.)	Serum Iron (µg./100 ml.)	% Fe abs. % of 5 mg. Dose	Plasma Proteins: Total (g./100 ml.)	Plasma Proteins: Albumin (g./100 ml.)	Ca (mg./100 ml.)	P (mg./100 ml.)	Alkaline Phosphatase (units)	Faecal Fat (g./day)	N_a (g./day)	Urinary Xylose (g./5 hr.)	Uropepsinogen units/24 hr.)	B_{12} Absorption (% of 0·5 µg. dose): Alone	B_{12} Absorption: With I.F.	B_{12} Absorption: With Carbachol	B_{12} Absorption: With Histamine
Subjects with oesophago-jejunostomy with by-passed stomach																					
63	M	11	Hepamino B_{12} since 1956	16·3	Normo-blastic	450	104	—	6·5	4·1	7·9	3·8	6	6·8	0·9	4·3	3·0	26·9	50·0	—	—
59	F	9	Hepamino	12·4	Megaloid	45	27	—	6·3	3·7	8·0	4·0	22	19·0	1·2	3·6	47	9·0	38·0	—	37·0
60	F	10	Hepamino	13·5	Megaloid	55	52	20	6·7	3·9	7·6	3·1	10	—	—	—	18	19·0	37·0	—	—
67	F	8	Hepamino	13·1	Normo-blastic	38	104	16	7·2	4·0	9·5	4·0	7	40·0	4·5	5·2	98	31·0	50·0	40·0	—
53	F	9	Hepamino Meglovite oral B_{12}	12·7	Normo-blastic	240	151	18·3	6·5	4·2	9·6	4·9	5	21·0	2·2	6·2	45	28·0	61·0	40·0	26·0
68	M	8	Hepamino Occasional B_{12} since 1957	14·5	Megaloid giant bands+	78	124	—	6·9	4·0	10·4	3·6	8	7·0 10·5	1·89 2·69	7·0	66	23·0	33·0	—	42·0
49	F	11	Hepamino B_{12} since 1953	15·0		860	88	—	—	—	—	—	—	—	—	—	13·5	19·0	46·0	10·0	—
43	F	8	Hepamino 1950-54, folic and oral B_{12} 1954, liver injections 1954-55	12·5	Megaloid giant bands+	78	124	6	5·6	3·7	9·0	3·6	2	10·8	1·8	4·7	17	5·0	29·0	44·0	17·0
51	F	7	Hepamino + B_{12} tablets for a time	12·0	Megalo-blastic	45	46	19·4	5·5	2·9	7·6	3·3	15	11·2	—	4·6	79	24·0	33·0	44·0	27·0
59	M	6	Folic acid 1956-57, later proteo-lysed liver	10·4	Megalo-blastic	50	168	—	6·1	4·0	9·8	4·8	9	8·7	1·4	—	25	31·0 pre-op.	55·0	—	—
after 2nd operation				14·4	Normo-blastic	180	—	—	—	—	—	—	—	—	—	—	—	17·0 post-op.	—	—	—
38	F	12	Hepamino B_{12} injection since 1952. A+D and Fe	13·7		1,300	110	—	6·6	4·1	8·4	3·1	17	4·5	1·6	7·1	117	24·0 pre-op. 42·4 post-op.	48·2	—	22·6
62	M	4	Hepamino	16·1	Normo-blastic	38	71	8	5·9	3·6	9·4	3·7	5	15·2	2·15	6·8	76	22·0	50·0	—	2·53
Subjects with oesophago-jejuno-gastrostomy																					
43	M	6 mths.	None	16·6	Normo-blastic	160	—	—	6·2	4·3	11·5	—	—	5·7	1·9	4·8	—	65	—	—	—
42	F	5	Liver extract Multiple vitamins	14·0		1,200	117	—	7·0	4·7	8·2	2·8	8	6·7	1·1	4·5	21	86	—	—	—

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developed anaemia three to eight years following operation and were given liver injections, folic acid, or vitamin B_{12}.

Other symptoms complained of were sore mouth and tongue in six, paraesthesiae in hands and feet in five, cramps in six, bone pain in three, which was accompanied by radiological evidence of osteoporosis in two, and oedema in two.

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Dy.	59	F	9	Hepamino	12·4	Megaloid	45	27	—	6·3	3·7	8·0	4·0	22	19·0	1·2	3·6	47	9·0	38·0	—	37·0
W.	60	F	10	Hepamino	13·5	Megaloid	55	52	20	6·7	3·9	7·6	3·1	10	—	—	—	18	19·0	37·0	—	—
C.[1]	67	F	8	Hepamino	13·1	Normoblastic	38	104	16	7·2	4·0	9·5	4·0	7	40·0	4·5	5·2	98	31·0	50·0	40·0	—
L.	53	F	9	Hepamino Meglovite oral B_{12}	12·7	Normoblastic	240	151	18·3	6·5	4·2	9·6	4·9	5	21·0	2·2	6·2	45	28·0	61·0	40·0	26·0
H.	68	M	8	Hepamino Occasional B_{12} since 1957	14·5	Megaloid giant bands+	78	124	—	6·9	4·0	10·4	3·6	8	7·0 10·5	1·89 2·69	7·0	66	23·0	33·0	—	42·0
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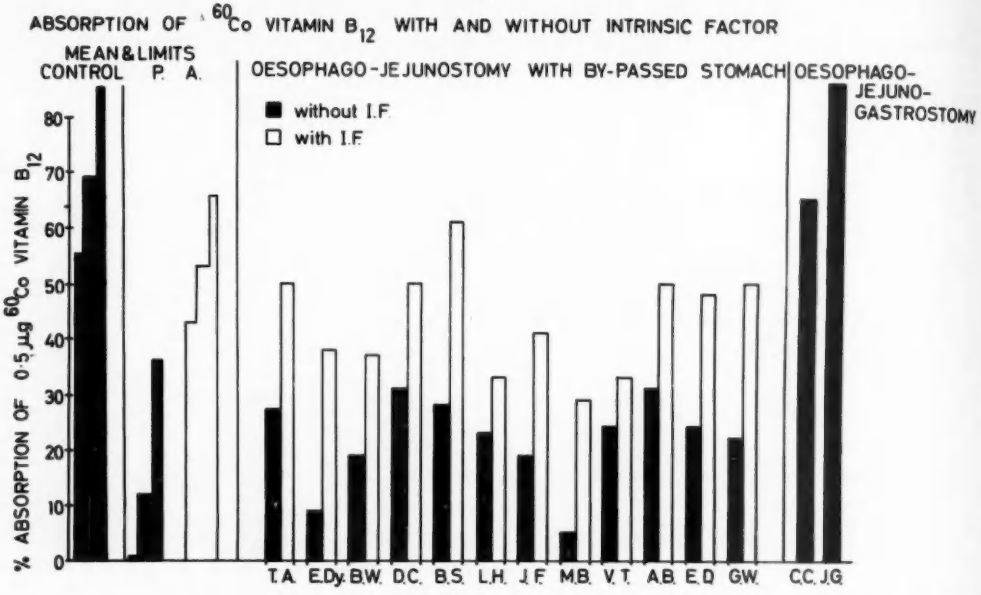

FIG. 2. *Percentage absorption from an oral dose of 0·5 µg. ^{60}Co vitamin B_{12} with and without intrinsic factor (I.F.) in 12 patients with oesophago-jejunostomy and by-passed stomach and two with oesophago-jejuno-gastrostomy.*

in none was anaemia severe at the time of observation. The lowest haemoglobin was 10·4 g. per 100 ml. Most of the values were within normal limits (Table I), although the majority of the blood films showed some macrocytosis. In two the bone marrow was megaloblastic, in four megaloid with many giant band forms, and in four normoblastic; one of these last four had been receiving parenteral vitamin B_{12} and another oral vitamin B_{12}. In the remaining two the sternal marrow was not examined because they were on adequate maintenance therapy with vitamin B_{12}. Prothrombin time was slightly prolonged in three of the eight in whom it was tested.

SERUM VITAMIN B_{12} The level of serum vitamin B_{12} was less than 100 $\mu\mu$g./ml. in all the patients except three who were receiving injections of vitamin B_{12} and one who was taking oral tablets of vitamin B_{12}.

ABSORPTION OF ^{60}CO-VITAMIN B_{12} Absorption of radioactive vitamin B_{12} was reduced in all the subjects. The values were all in the range for pernicious anaemia although the mean absorption for the group (22%) was somewhat greater than the mean for pernicious anaemia (Fig. 2).

The addition of intrinsic factor enhanced absorption in all, although in two (V.T. and L.H.) there was only a 9% and 10% difference, respectively, which is doubtfully significant. In seven only of these patients was the preparation of intrinsic factor as effective in restoring the absorption towards normal as it was in subjects with pernicious anaemia.

Carbachol was given to five subjects in the test of absorption of vitamin B_{12}. In one, less of the vitamin was absorbed than without carbachol stimulation. In two the absorption was greater than that obtained with the addition of intrinsic factor and in the remaining two there was a doubtful enhancing effect.

Histamine stimulation was combined with a test of vitamin B_{12} absorption in seven subjects. In four there was no difference between absorption with and without histamine stimulation; in the remaining three there was an increase in absorption similar to that obtained with intrinsic factor.

SERUM IRON In contrast to the reduced levels of serum B_{12} the serum iron was within normal limits in all except three and in one only was it notably low (27 µg./100 ml.).

IRON ABSORPTION This was studied in five subjects. The percentage values for iron absorption were all below the mean of 25% of a 5-mg. dose of iron

obtained in control non-anaemic subjects. In two in whom the serum iron was low (B.W. and V.T.) absorption was not as high as is usual in iron deficiency although it came within the control range.

FAT AND NITROGEN EXCRETION These were studied in 10 of the 12 patients. Steatorrhoea was a feature in all except one. The exception (E.D.) had been observed to have steatorrhoea when studied in another hospital in 1952, at which time she had been started on parenteral vitamin B_{12}.

The amount of fat excreted a day varied from 4·5 to 40 g. with a mean of 14·5 g. in 24 hours. Nitrogen excretion exceeded 2 g. a day in four subjects.

It was noteworthy that the patient showing the grossest degree of steatorrhoea and negative nitrogen balance was the only one in whom special care had been taken not to divide the vagi at operation.

UROPEPSINOGEN EXCRETION Seven of the 12 subjects excreted less than 50 units of uropepsinogen in 24 hours. The remaining five showed normal excretion of uropepsinogen.

XYLOSE ABSORPTION TEST This indicated normal absorption of xylose in the eight subjects tested.

BLOOD CHEMISTRY The total plasma proteins were less than 6·5 g. per 100 ml. in five of the subjects, in three they were under 6 g. per 100 ml. In all, the albumin fraction was less than 4·4 g. per 100 ml. and in eight it was 4 g. per 100 ml. or less.

Serum calcium was reduced to 9 mg./100 ml. or less in seven subjects but this was certainly at least in part due to the hypoproteinaemia. The alkaline phosphatase was slightly increased in three subjects.

HISTOLOGY In six subjects biopsy of the jejunum was taken through the oesophagoscope. Five were normal. The sixth (E.Dy) showed loss of villi and a jejunitis comparable with that seen in idiopathic steatorrhoea.

Three subjects had sufficient symptoms and discomfort to warrant re-operation in order to put the stomach back into continuity with the gastro-intestinal tract, and to convert the condition to an oesophago-jejuno-gastrostomy. The first (A.B.) was found at operation to have a small portion of the stomach intact (about 6 cm. in length). Part of the cardia had been removed at the original operation. Biopsy of the remnant showed a completely normal mucosa (Fig. 3). Jejunal biopsy, however, showed some mild chronic inflammatory change and some pigmentation with lipofuchsin in the muscularis mucosae.

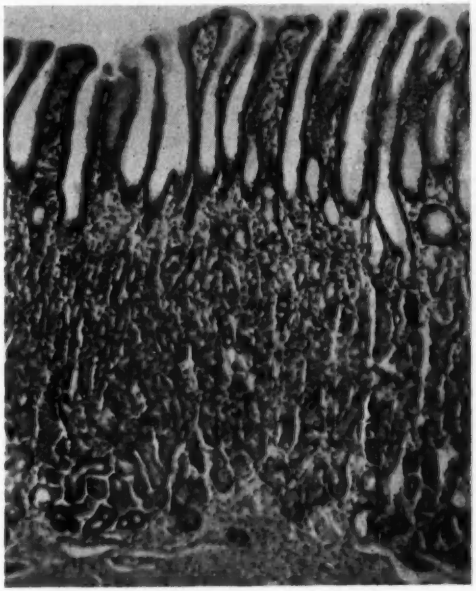

FIG. 3 *Histology of gastric mucosa (patient A.B.) six years after the original by-pass operation.*

The second and third patients (E.D. and G.W.) showed a superficial gastritis with preservation of the normal glandular tissue but normal histology of the jejunum.

RESULTS OF RE-OPERATION A.B. received no treatment with vitamin B_{12} after operation but he improved clinically and put on weight. When seen six months later his serum B_{12} was 180 $\mu\mu$g./ml. and his count was normal. A single test of absorption of labelled vitamin B_{12} did not, however, reveal any improvement in absorption. It would clearly be desirable to repeat this test. Attempts to obtain gastric juice after operation were unsuccessful. The second patient (G.W.) was unfortunately found to have a bronchial neoplasm which was removed at the time of his re-operation. He was never sufficiently well to be submitted to any adequate post-operative studies.

The third patient (E.D) was studied more fully. Nine days after the operation the absorption of labelled B_{12} had improved from 24 to 42%, *i.e.*, to a value comparable to that found previously with the addition of intrinsic factor. A sample of this patient's gastric juice obtained through a gastrostomy a few days after operation was acid, and 100 ml. of the juice improved the absorption of ^{60}Co-vitamin

B_{12} in a patient with pernicious anaemia from 12 to 78%, indicating that it contained active intrinsic factor.

RESULTS IN PATIENTS WITH OESOPHAGO-JEJUNO-GASTROSTOMY

Two patients (J.G. and C.C.) were studied five years and six months respectively after the operation of oesophago-jejuno-gastrostomy. Both had had severe symptoms dating back to infancy, necessitating feeding by gastrostomy for periods of years, and many attempts to relieve their peptic stricture of the oesophagus by dilatation or less drastic surgical procedure. The operation of oesophago-jejuno-gastrostomy was of great benefit to both.

Like the first group they both suffered from diarrhoea after operation although one only (J.G.) had it severely. The diarrhoea gradually settled after a year, but this patient lost about 7 kg. of weight, this being her main post-operative complaint. She had treated herself with multiple vitamin preparations plus calcium and liver extract since operation. The other patient had no specific therapy.

Blood counts and bone marrow of both these patients were normal. The subject who had been having injections of liver extract had a serum B_{12} of 1,500 $\mu\mu$g./ml., in the other it was 160 $\mu\mu$g./ml. In contrast to the patients with a by-passed stomach, absorption of ^{60}Co-vitamin B_{12} was normal in both.

There was a borderline steatorrhoea in both, the plasma proteins were low in one, probably reflecting his long period of malnutrition before operation; in the other the proteins were normal but the serum calcium was reduced.

Uropepsinogen excretion was measured in one only and it was reduced.

A biopsy was taken from the jejunal loop through an oesophagoscope and in both subjects the appearance of the mucosa was normal.

DISCUSSION

The patients were seen in the course of a review of the results of oesophago-jejunostomy with by-pass of the stomach for oesophageal stricture. They had received a variable amount of antianaemic treatment and it would not have been practicable to impose a control period without therapy, even had this been justifiable on other grounds. The surprising thing is that the majority of the patients nevertheless showed a condition which could most simply be described as a latent or subclinical pernicious anaemia. The level of vitamin B_{12} in the serum was in the pernicious anaemia range except for three patients who were receiving injections of vitamin B_{12} and one who was taking oral tablets of vitamin B_{12}. The blood films were mostly macrocytic and the marrow films were megaloblastic or megaloid in the majority of cases. Absorption of radioactive vitamin B_{12} was reduced in all cases to the levels found in pernicious anaemia and in all it was improved by the simultaneous administration of intrinsic factor, although not always as effectively as in pernicious anaemia.

The reason for the development of vitamin B_{12} deficiency is not at all clear. It does not seem to have been due to defective intestinal absorption. Although steatorrhoea was present, often in considerable degree, intrinsic factor was effective in enhancing absorption of vitamin B_{12}. Xylose was absorbed normally and jejunal biopsy usually gave normal results.

The low level of uropepsinogen excretion in more than half the patients might suggest that the by-passed stomach was inactive and failed to secrete intrinsic factor. Clearly, the gastric phase of gastric secretion should have been abolished by the operation. The psychic secretion was abolished or diminished because the vagi were usually densely bound up in the peri-oesophagitis associated with the stricture and therefore were almost certainly divided at operation. Nevertheless, in one case it was believed that the vagi were in part preserved. The intestinal phase of gastric secretion was likely to have been reduced by the side-tracking of the duodenum though some regurgitation of food into the duodenum may occur in these cases. However, it would seem probable from the work of Johnston and Welbourn (1958) and Plzak, Price, and Woodward (1958), who have carried out an identical operation in dogs, that gastric juice is secreted and enters the intestine in these cases.

Quite small amounts of intrinsic factor are probably sufficient to prevent pernicious anaemia, as shown by the rarity of its occurrence after partial gastrectomy and its absence in more than half the cases of complete achlorhydria (Callender, Retief, and Witts, 1960). The histology of the gastric mucosa was normal or near normal in the three cases where it was possible to examine it, and in one of them the gastric juice was shown to contain intrinsic factor after restoration of the continuity of the stomach with the gastrointestinal tract. Finally, there is the fact that stimulation of the by-passed stomach by carbachol and histamine was not always effective in increasing absorption of vitamin B_{12}. Suppression of gastric secretion is therefore unlikely to be the main explanation of the development of vitamin B_{12} deficiency in these patients, although it may play some part.

It would seem more likely that the normal conditions for the complexing of vitamin B_{12} and

intrinsic factor before absorption are not satisfied when the intrinsic factor is not delivered until some little way down the jejunum instead of in the stomach. In these circumstances vitamin B_{12} may become bound to substances which prevent its union with intrinsic factor, or intrinsic factor may be inactivated before it meets the vitamin B_{12}. Unfortunately, no experiments were carried out in which the tracer dose of radioactive vitamin B_{12} was given together with a vitamin B_{12}-free meal. It has recently been shown in this laboratory that after partial gastrectomy radioactive vitamin B_{12} is absorbed better when given with food than when given alone, and similar conditions might prevail in these patients with by-passed stomachs (Deller, Germor, and Witts, 1961).

In contrast to the frequency of vitamin B_{12} deficiency in these cases is the rarity of iron deficiency. The absorption of iron was impaired, but not to the degree seen in idiopathic steatorrhoea. Only three of the patients were women who were still menstruating so that the demands for iron were not great. In view of the considerable degree of steatorrhoea it is not surprising that serum calcium levels tended to be low though serum phosphorus and phosphatase levels were normal. Serum albumin also tended to be low but the falls in calcium and albumin were not closely correlated. Osteomalacia was not seen but osteoporosis occurred in two women. Although in one this may have been only an aggravation of the common tendency to osteoporosis seen after the menopause, the other (E.D.) was the youngest woman in the series and the osteoporosis appeared to be attributable solely to the operation. She developed anaemia and gross steatorrhoea, both of which appeared to improve on parenteral vitamin B_{12} therapy.

The observations on the two subjects with oesophago-jejuno-gastrostomy indicate the superiority of this operation in that the absorption of vitamin B_{12} was normal, and there was little if any steatorrhoea.

SUMMARY

Twelve subjects in whom the operation of oesophago-jejunostomy was performed, leaving the stomach *in situ*, were studied four to 12 years after the operation.

All showed considerable metabolic disturbance, the main feature being steatorrhoea and inability to absorb vitamin B_{12}.

Gastric secretion may be suppressed but the more likely explanation of the poor absorption of vitamin B_{12} is disturbance of the normal conditions for the complexing of vitamin B_{12} and intrinsic factor.

The operation has now been abandoned in favour of oesophago-jejuno-gastrostomy. Two patients in whom this operation had been performed showed normal absorption of vitamin B_{12}.

We are indebted to Dr. G. H. Spray for estimations of vitamin B_{12} in the serum, Mr. J. R. P. O'Brien for some of the biochemical estimations, Dr. W. C. D. Richards for histological reports, and Miss B. M. Mallett and Mr. S. W. Perry for technical assistance. The work was in part aided by a grant to Professor L. J. Witts from the Medical Research Council.

REFERENCES

Aitken, M. A., Spray, G. H., and Walters, G. (1954). Gastric pepsin and the excretion of uropepsinogen in anaemia. *Clin. Sci.*, **13**, 119-126.

Anson, M. L. (1938). The estimation of pepsin, typsin, papain, and cathepsin with hemoglobin. *J. gen. Physiol.*, **22**, 79-89.

Bradley, J. E. (1957). Quoted by Mollin, D. L., Booth, C. C., and Baker, S. J. *Brit. J. Haemat.*, **3**, 412-428.

Callender, S. T., Mallett, B. J., and Smith, M. D. (1957). Absorption of haemoglobin iron. *Ibid*, **3**, 186-192.

——, Retief, F. P., and Witts, L. J. (1960). The augmented histamine test with special reference to achlorhydria. *Gut*, **1**, 326-336.

——, Turnbull, A., and Wakisaka, G. (1954). Estimation of intrinsic factor of Castle by use of radioactive vitamin B_{12}. *Brit. med. J.*, **1**, 10-12.

Deller, D. J., Germor, H., and Witts, L. J. (1961). Effect of food on absorption of radioactive vitamin B_{12}. *Lancet*, **1**, 574-577.

Johnston, I. D. A., and Welbourn, R. B. (1958). The influence of the secretory function of the stomach on nutrition in the dog. *Brit. J. Surg.*, **46**, 163-169.

Kamer, J. H. van de, Bokkel Huinink, H. ten, and Weyers, H. A. (1949). Rapid method for the determination of fat in feces. *J. biol. Chem.*, **177**, 347-355.

Perry, S. (1960). Unpublished data.

Pizak, L., Price, L., and Woodward, E. R. (1958). The effect of complete exclusion of the stomach on fat and nitrogen absorption. *Surgery*, **44**, 299-392.

Retief, F. P. (1959). *A study of achlorhydria and its relation to anaemia.* Thesis submitted for D. Phil., Oxon.

Spray, G. H. (1955). An improved method for the rapid estimation of vitamin B_{12} in serum. *Clin. Sci.*, **14**, 661-667.

——, and Witts, L. J. (1958). Results of three years' experience with microbiological assay of vitamin B_{12} in serum. *Brit. med. J.*, **1**, 295-298.

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A clinical appraisal of the treatment of chronic duodenal ulcer by vagotomy and gastric drainage operation

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SYNOPSIS The authors have followed up 100 patients upon whom a vagotomy plus a drainage operation was performed for chronic duodenal ulceration by one of them (H.C.E.) at King's College Hospital during the 11-year period 1948-1958.

DATA

All the patients entered the hospital because of chronic duodenal ulceration. Eighty-one were men and 19 were women. Their average age at the time of operation was 39·8 years, with a range of 14 to 74 years, and the length of symptoms before surgery averaged 10·2 years, with a range of one to 30 years. Previous ulcer complications had occurred in 37 of the patients as follows:—Bleeding (16), obstruction (13), and perforation (8). Vagotomy was followed by gastroenterostomy in 90 patients and by pyloroplasty in 10 (Table I).

All patients underwent gastric acid studies before operation, including resting free and total acid determinations and insulin response (Hollander, 1946, 1948). These tests were then repeated post-operatively, usually within two weeks of operation, or occasionally a number of months later, at a time of a re-admission. The acid studies of a few of the cases are not reported, either because the tests were deemed to be unsatisfactory, or because the records are lost.

TABLE I

SUMMARY OF RESULTS

Cases	100
Male	81
Females	19
Mean age (yr.)	39·8 (14-74)
Mean length of symptoms (yr.)	10·2 (1-30)
Total ulcer complications before surgery	37%
Bleeding	16%
Obstruction	13%
Perforation	8%
Pre-operative acid studies	
Resting free acid (mEq./l.) in 89 cases	46 (0-110)
Insulin response in 88 cases	87 positive
Type of operation performed with vagotomy	
Pyloroplasty and gastroenterostomy	90
Pyloroplasty	10
Complications and mortality (%)	
Severe	1
Mild	15
Mortality	1
Post-operative acid studies	
Resting free acid (mEq./l.) in 82 cases	10 (0-60)
Insulin response in 82 cases	13 positive
Mean length of follow-up (yr.)	4·9 (2-11)
Success of surgery in patients' opinion	96% yes
Full day's work	96% yes
Recurrent ulcer	5%
Weight change (%)	
Gain	23
Loss	13
Gastrointestinal symptoms (%)	
Dumping	6
Diarrhoea	15
Other	15

OPERATIVE TECHNIQUE

A right paramedian incision is made from the left xphisternocostal angle to the umbilicus, and the diagnosis of duodenal ulceration confirmed. The left lobe of the liver is retracted upwards and to the right (there is rarely need to divide the coronary ligament) and the vagus nerves exposed by incising the peritoneum and fascia propria over the lower end of the oesophagus, beginning the incision to the left of the oesophagus. Further exposure is obtained by gentle traction on a length of Paul's tubing passed around the oesophagus. The left (anterior) vagus is usually visible, but on occasion needs to be located with the finger. The right (posterior) branch is located manually and brought into view by hook retraction. One to one and a half inches of each

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nerve is resected, and both proximal and distal ends ligated with fine thread. Any residual smaller branches are recognized by finger palpation, and divided without ligature. Considerable time and care is taken to ensure that all vagal nerve tissue is divided, although, as will be seen, this was not always achieved.

The gastroenterostomy is made just posterior to the greater curvature of the stomach, and placed as near to the pylorus as is technically suitable, and the stoma made large enough to admit three fingers. It is isoperistaltic, with no loop. If a pyloroplasty is employed as the drainage procedure, an adequate stoma is ensured by making the incision through the antro-duodenal wall two inches in length, and centred at the pylorus. The patients do not normally receive intravenous therapy or blood replacement during or after operation.

After discharge from hospital all the patients have been seen at three-monthly intervals in the out-patient surgical clinic during the first year, and at least once during the second year. From then on follow-up has continued in a somewhat haphazard way, sometimes by mail and sometimes by clinic visits. At the time of this evaluation all patients were sent a questionnaire. In addition, 39 of the patients were interviewed.

COMPLICATIONS AND MORTALITY OF SURGERY

Complications after operation were uncommon (Table II). There was one case of pulmonary embolus,

TABLE II

COMPLICATIONS OF SURGERY

Severe	
Pulmonary embolus	1%
Mild	
Stomal obstruction	4%
Wound sepsis	1%
Pneumonia or atalectasis	8%
Thrombophlebitis	1%
Total	15%
Mortality of surgery in 100 cases from pulmonary embolus	1%

which resulted in the only death in the series. Other complications were mild, and included four cases of transitory stomal obstruction, one case of superficial wound sepsis, eight cases of pneumonia or atelectases (all successfully treated by physiotherapy, coughing, and/or antibiotics), and one case of femoral thrombophlebitis.

ACID STUDIES AND RECURRENCE OF ULCERATION

The average resting free acid in 89 pre-operative patients was 46 mEq./l. with a range of 0 to 110 mEq./l. (Table III). This can be compared with an average resting free acid in 82 post-operative patients of 10 mEq./l. with a range of 0 to 60 mEq./l.

TABLE III

ACID STUDIES

Cases	*Pre-operative* 89	*Post-operative* 82
Resting free acid (mEq./l.)	46 (0-110)	10(0 -60)
Insulin response (%)	99	16

Of 82 patients, 99% had a positive pre-operative insulin response, while only 16% had a positive post-operative insulin response.

The relation between the post-operative insulin response and ulcer recurrence is interesting and significant (Table IV). In none of the 69 patients

TABLE IV

INSULIN RESPONSE AS MEASURE OF EFFECTIVENESS OF SURGERY

Post-operative	*Cases*	*No.*	*%*
Insulin Response		*Recurrent Ulcers*	*Recurrence*
Negative	69	0	0
Positive	13	5	38

with a negative post-operative insulin response has there thus far been evidence of recurrent ulceration. Of the 13 cases with a positive post-operative insulin response, five cases (38%) have shown evidence of recurrent ulceration.[2] Their post-operative histories are briefly as follows:—

CASE 1 This 34-year-old man entered King's College Hospital with a four-year history of intractable duodenal ulceration. Acid studies revealed a pre-operative resting free acid level of 60 mEq./l. Post-operative resting free acid was recorded at 40 mEq./l. and the insulin response was strongly positive. Ulcer symptoms recurred six months port-operatively. Subtotal gastrectomy was performed and the patient has now been well for five years.

CASE 2 This 28-year-old man entered King's College Hospital with a five-year history of intractable duodenal ulceration. Acid studies revealed a pre-operative resting free acid level of 50 mEq./l. Post-operative resting free acid was recorded at 20 mEq./l. and the insulin response was strongly positive. Three years post-operatively the patient noted recurrent ulcer symptoms. He was treated with antacids and diet and is doing well one and a half years after recurrence of symptoms.

CASE 3 This 29-year-old man entered King's College Hospital with a six-year history of intractable duodenal

[2]In one of them the drainage operation was a pyloroplasty.

ulceration. Acid studies revealed a pre-operative resting free acid level of 70 mEq./l. Post-operative resting free acid was recorded at 30 mEq./l. and the insulin response was strongly positive. Two years post-operatively he noted recurrence of ulcer symptoms and gastro-duodenal bleeding; he was treated with antacids and diet and is doing well five years after recurrent trouble.

CASE 4 This 26-year-old man entered King's College Hospital with a history of intractable duodenal ulceration. Acid studies revealed a pre-operative resting free acid level of 60 mEq./l. Post-operative resting free acid was recorded at 60 mEq./l. and the insulin response was strongly positive. One and a half to two and a half years post-operatively, the patient noted recurrent ulcer symptoms and gastroduodenal bleeding; he was treated with diet and antacids, and is now doing fairly well five years after his last recurrent symptoms.

CASE 5 This 31-year-old man entered King's College Hospital with a five-year history of intractable duodenal ulceration. Acid studies revealed a pre-operative resting free acid level of 50 mEq./l. Post-operative resting free acid was recorded at 20 mEq./l. and the insulin response was moderately positive. One year after surgery he noted recurrence of ulcer symptoms. He was treated with diet and antacids and is doing fairly well two and a half years after recurrence.

RESPONSES TO ENQUIRIES

WAS THE OPERATION A SUCCESS? All patients were asked whether they considered their operation a success, and 96 of the 99 living patients said yes. The three patients who said no all had recurrent ulcers. (The two additional patients with recurrent ulcer disease thought their operation was a success, because they felt much better and more able to control their symptoms with diet and medication.)

FULL DAY'S WORK Of the 99 living patients, 96 are now doing a full day's work—as much or more work than they were doing pre-operatively at a time when they felt well. There are three patients who are unable to do a full day's work, two because of recurrent ulcer symptoms and one because of 'general ill health', as was present before his operation.

SYMPTOMS As regards post-operative symptoms (Table V), 6% of the patients reported discomforts suggestive of the dumping syndrome (5% very mild, 1% moderate). In the 'mild' cases the symptoms were vasomotor in type, and in the one 'moderate' case bilious vomiting was the predominant feature. In none were the symptoms severe enough to keep the patient from doing a full day's work (five gastroenterostomy, one pyloroplasty).

TABLE V

POST-OPERATIVE SYMPTOMS RELATED TO SURGERY

	Mild (%)	*Moderate* (%)	*Severe* (%)
Dumping	5	1	0
Diarrhoea	13	2	0
Flatulence or OCC indigestion	15	0	0

Fifteen had diarrhoea (13 mild, 2 moderate). In no case was the diarrhoea severe enough for the patient to consider this a serious problem, or to keep the patient from doing a full day's work. (Two of the mild cases had a pyloroplasty.)

Fifteen of the patients had various complaints of flatulence and indigestion, all of a mild degree (one pyloroplasty, 14 gastroenterostomy).

WEIGHT Twenty-three per cent. of the patients gained weight. The average gain was 14 lb., with a range of 2 to 56 lb. Thirteen of the patients lost weight. The average loss was 9 lb., with a range of 2 to 24 lb. (Table VI).

TABLE VI

CHANGE IN WEIGHT SINCE SURGERY

Gain in weight		23%
Average gain	14 lb.	
Range	2 to 56 lb.	
Loss in weight		13%
Average loss	9 lb.	
Range	2 to 24 lb.	

ANAEMIA Only one patient has been noted to be anaemic. This patient has required iron to maintain her haemoglobin at 80%.

DISCUSSION

A recurrence rate of 5% with a 4·9 year average follow-up compares favourably with the recurrence rate after subtotal gastrectomy or after vagotomy alone. Statistics compiled by the Committee on Peptic Ulcer of the American Gastroenterological Association (Sandweiss *et al.*, 1952) revealed a recurrence of ulcer symptons in 4·3% of patients after subtotal gastrectomy. This is similar to the recurrence rate of 4·2% after subtotal gastrectomy recently reported by Armstrong and Penick (1960). Brooks and Moore (1953) found a recurrence rate of 13% after vagotomy alone and Sandweiss *et al.* (1952) have reported even higher recurrence rates following this procedure. The results of vagotomy and a gastric drainage procedure, however, do not match the extraordinarily low incidence of recurrent ulceration associated with hemigastrectomy and

vagotomy. Farmer and Smithwick (personal communication) and Edwards, Herrington, Stephenson, Carlson, Phillips, Cate, and Scott (1957) found a recurrence rate after this procedure of 0·9% and 0% respectively.

The relationship of recurrent ulcer disease with the completeness of vagotomy as indicated by the insulin response test is significant. No case in this series with a negative insulin response post-operatively has thus far developed a recurrent ulcer, while 38% of the cases with a positive post-operative insulin response have suffered from recurrent disease.

Undesirable side-effects following vagotomy and a gastric drainage procedure are neither frequent nor severe. Only 6% of our patients developed symptoms consistent with the dumping syndrome and in only one case was this of even moderate severity. These results compare favourably with a reported 11% to 33% incidence of the dumping syndrome after subtotal gastrectomy (Hayes, 1955; Jordan, 1958; Armstrong and Penick, 1960) and of 23·1% after hemigastrectomy and vagotomy (Edwards *et al.*, 1957).

The absence of side-effects after vagotomy and a drainage procedure is also reflected by weight statistics, which show that more of our patients gained than lost weight after operation. Even more significant is the fact that of the 13 patients who lost weight only two lost more than one stone, and in none was weight loss a serious problem. In contrast, reported results following subtotal gastrectomy indicate an approximate 40% incidence of weight loss (Muir, 1949; Ivy, Grossman, and Bachrach, 1950); in about 18% to 22% (Muir, 1949; Armstrong and Penick, 1960), the weight loss exceeded one stone. Excessive weight loss may also be a problem after hemigastrectomy and vagotomy. Farmer and Smithwick (personal communication) recently found that a moderate or severe weight loss occurred in 3·2% of their patients after this operation.

Vagotomy and a gastric drainage procedure is associated with a high incidence of post-operative diarrhoea. It occurred in 15 of our patients, but in only two cases was it more than mild in degree, and in none was it so severe as to incapacitate. Reported results following subtotal gastrectomy reveal a lesser incidence of diarrhoea, which is usually mild in degree (Sandweiss *et al.*, 1952). (The incidence of post-operative symptoms was on a similar scale after pyloroplasty as after gastroenterostomy, but the number of patients submitted to the former are too small to assess the relative merits of the two procedures from the clinical standpoint.)

Finally, the operation of vagotomy and gastric drainage is usually technically easy and quick to perform and thus avoids the complications, immediate and remote, which are inherent in the operation of partial gastrectomy. The absence of severe operative complications, the low operative mortality coupled with an incidence of side effects and recurrent ulceration, which compares favourably with alternative forms of surgical treatment, strengthen our belief that this procedure amply justifies itself as a method of treatment for chronic duodenal ulceration.

SUMMARY AND CONCLUSIONS

1 One hundred patients with chronic duodenal disease treated by vagotomy and a gastric drainage operation are reviewed.

2 Vagotomy and a gastric drainage operation is usually an easier and safer procedure than partial gastrectomy, and is associated with a lower operative mortality and morbidity.

3 Recurrent symptoms occurred only in those patients in whom the post-operative insulin response indicated that vagotomy had been incomplete. It is therefore essential that vagotomy should be complete.

4 The long-term post-operative side effects of this procedure are less frequent and less debilitating than those associated with operations for chronic duodenal ulceration which entail removal of part of the stomach.

APPENDIX

RESTING FREE AND TOTAL ACID STUDIES AND INSULIN RESPONSE

These tests are carried out after the patient has been starved from 6 p.m. to 9 p.m. A Ryle's tube is passed into the stomach through the nose, and 5 to 10 ml. of gastric contents withdrawn at hourly intervals for 12 hours. Fifteen to 20 units of regular insulin are then given intravenously. After 15 minutes, further quarter hourly specimens of gastric contents are withdrawn for one hour. The Ryle's tube is then removed and the patient given 50 grams of glucose.

Free and total acid levels are determined on all samples by titration using thymol blue as indicator.

REFERENCES

Armstrong, R. A., and Penick, R. M. (1960). Surgical treatment of benign peptic ulcer. *Ann. Surg.*, **152**, 109-118.

Brooks, J. R., and Moore, F. D. (1953). Vagotomy for duodenal ulcer. A final survey after 10 years. *New Engl. J. Med.*, **249**, 1089-1097.

Edwards, L. W., Herrington, J. L. Jr., Stephenson, S. E. Jr., Carlson, R. I., Phillips, R. J. Jr., Cate, W. R. Jr., and Scott, H. W. Jr. (1957). Duodenal ulcer: Treatment by vagotomy and removal of the gastric antrum. *Ann. Surg.*, **145**, 738-752.

Hayes, M. A. (1955). The dietary control of the postgastrectomy "dumping syndrome". *Surgery*, **37**, 785-793.

Hollander, F. (1946). The insulin test for the presence of intact nerve fibers after vagal operations for peptic ulcer. *Gastroenterology*, **7**, 606-614.

—— (1948). Laboratory procedures in the study of vagotomy (with particular reference to the insulin test). *Ibid*, **11**, 419-425.

Ivy, A. C., Grossman, M. I., and Bachrach, W. H. (1950). *Peptic Ulcer*. Blakiston, Philadelphia.

Jordan, G. L. (1958). Treatment of the dumping syndrome. *J. Amer. med. Ass.*, **167**, 1062-1066.

Muir, A. (1949). Postgastrectomy syndromes. *Brit. J. Surg.*, **37**, 165-178.

Sandweiss, D. J., Barborka, C. J., Bockus, H. L., Ivy, A. C., Jordan, S. M., Miller, T. G., Palmer, W. L., Wangensteen, O. H., Ruffin, J. M., Aaron, A. H., Hollander, F., Thomas, J. E., Walters, W., Moore, F. D., Winkelstein, A., Brooks, F. P., and Lorge, I. (1952). Report of the Committee on Surgical Procedures of the National Committee on Peptic Ulcer of the American Gastroenterological Association on Study of Vagotomy, Study of Gastric Resection, Comparative Study of Vagotomy and Gastric Resection. *Gastroenterology*, **22**, 295-499.

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Gastric acid secretion and mucosal appearances in Addison's disease and hypopituitarism

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SYNOPSIS Studies of gastric acid secretion and mucosal appearances have been made in a group of 14 patients suffering from hypopituitarism. Achlorhydria was found in six of the patients suffering from Addison's disease but in only one patient suffering from hypopituitarism. In both groups the mean gastric secretion of hydrochloric acid was considerably lower than in a group of control subjects and replacement therapy with cortisone and DOCA in Addison's disease and cortisone and thyroid extract in hypopituitarism failed to restore gastric function to normal. A constant correlation was not found between gastric acid secretion and mucosal appearances.

Since Grawitz (1907) reported that the gastric acid secretion in Addison's disease was depressed various other workers have found achlorhydria in approximately half of such patients (Rowntree and Snell, 1931; Sorkin, 1949; Soffer, 1956). Card and Sircus (1958) pointed out, however, that no consistent changes in gastric acid secretion had been observed in Addison's disease using the augmented histamine secretion test (Kay, 1953). Hypochlorhydria or achlorhydria is also said to be a feature of hypopituitarism (Escamilla and Lisser, 1942; Soffer, 1956), and Card and Sircus (1958) have reported one patient suffering from hypopituitarism who had achlorhydria in response to the maximal histamine secretion test and another in whom the output of hydrochloric acid did not rise after histamine stimulation.

Feyrter and Klima (1952) noted that achlorhydria in Addison's disease may be associated with extensive gastric atrophy but apart from this observation very few studies have been made of the histology of the gastric mucosa in this disease or in hypopituitarism. Animal investigations, however, suggest that in such conditions the gastric mucous membrane undergoes atrophy and that there may be a diminution in the number of parietal cells. Haeger, Jacobsohn, and Kahlson (1953) found atrophy of the gastric mucosa in cats after removal of either the pituitary or the adrenals and concluded that the maintenance of the mucosa was a function of the pituitary gland and that this was dependent at least partially on the adrenal glands. Other workers state that in adrenalectomized (Baker and Bridgman, 1954) or hypophysectomized (Baker and Abrams, 1954) rats the zymogenic cells of the gastric mucosa are reduced in size and degranulated but there is relatively little structural change in the parietal cells. Friedman (1953), on the other hand, found that the parietal cell count was reduced by 50% in the gastric mucosa of rats that had been subjected to hypophysectomy.

Card and Marks (1960), using the modified augmented histamine secretion test (Kay, 1953; Card and Sircus, 1958), have shown that the output of hydrochloric acid by the stomach can be related to the parietal cell mass. Most of the previous reports on gastric secretion in Addison's disease and hypopituitarism have been based on less reliable and less exact methods. In view of this and because of the paucity of reports on gastric histology in these patients, it was decided to reinvestigate the question of gastric secretion by means of the augmented histamine secretion test and to correlate the results with the appearances of the gastric mucosa obtained by peroral biopsy.

MATERIALS AND METHODS

Fourteen patients suffering from Addison's disease, with an average age of 43 years, and 10 patients suffering from hypopituitarism with an average age of 52 years, were included in the study and they represented a successive series of patients who presented for admission to hospital or review at the endocrine clinic. These patients all had the classical features of Addison's disease or hypopituitarism and the diagnosis was confirmed by the usual laboratory investigations which included, when necessary, the measurement of urinary ketosteroids and

ketogenic steroids, a glucose tolerance test, an insulin sensitivity test, radioactive iodine studies, and an A.C.T.H. stimulation test. In the 14 Addisonian patients the disease was considered to be of tuberculous origin in six (Table I, Cases 3, 4, 5, 7, 9, and 11) and of the idiopathic variety in the remaining eight. Three were untreated or had received treatment for only a few days; the remaining 11 had received replacement therapy in the form of cortisone for periods varying from four to seven years and all except Case 6 (Table I) had received DOCA in addition, often for much longer periods.

All of the patients suffering from hypopituitarism were women. The disease followed surgical removal of a chromophobe adenoma from the pituitary in three (Table II, Cases 2, 3, and 6) but was considered to be consequent upon postpartum haemorrhage in five of the remaining seven patients (Cases 1, 4, 5, 7, and 10). In two (Cases 8 and 9), there was no obvious cause. Three of the 10 patients were untreated at the time of investigation but the remaining seven had received cortisone for periods varying from six months to eight years, supplemented by thyroid extract in most cases.

In each case the modified histamine secretion test (Kay, 1953; Card and Sircus, 1958) was carried out. After histamine stimulation the gastric juice was collected by continuous suction for the next hour and achlorhydria was considered to be present when the *p*H did not fall below 6. Gastric biopsies were performed with a Wood's gastric biopsy tube or the biopsy capsule of Crosby and Kugler (1957). The tip of the instrument was positioned in the body of the stomach under fluoroscopic control in every instance except one (Table II, Case 10).

RESULTS

GASTRIC SECRETION IN ADDISON'S DISEASE The relevant clinical and laboratory data are shown in Table I. Achlorhydria was found in five of the 14 patients and in one further patient (Case 5) no acid was found by titration but the *p*H fell to 5·5. The average secretion of hydrochloric acid in the post-histamine hour was 5 mEq. for the whole group (5·9 mEq. for men and 3·5 mEq. for women). The treated group had an average output of 3·1 mEq. of hydrochloric acid in the post-histamine hour. The untreated group contained only three representatives, but it is of interest to note that it included the patient with the highest secretion of all.

GASTRIC SECRETION IN HYPOPITUITARISM Details of the cases are shown in Table II. There was only one instance of complete achlorhydria. This patient was re-examined after six months' treatment with cortisone and achlorhydria was still present. The average output of hydrochloric acid in the post-histamine hour for the whole group was 6·6 mEq.

The mean values for gastric acid secretion in the group of patients suffering from Addison's disease and the group suffering from hypopituitarism are shown in Table III. It will be seen that in both groups the figures are considerably lower than those obtained from control subjects (Card and Sircus, 1960). In neither group was there any obvious correlation between the duration of the disease or the duration of treatment and the changes in acid secretion.

GASTRIC BIOPSIES Specimens were obtained from 12 patients with Addison's disease and from nine patients with hypopituitarism. They were fixed in corrosive-formol and stained with haematoxylin and eosin, also by Zimmermann's method for the differential staining of gastric mucosa, as modified by Marks and Drysdale (1957).

Addison's disease Eight of the 12 patients had abnormal gastric mucosa. In one patient (Case 1) there was glandular atrophy, *viz.*, complete absence of body glands and isolated parietal cells, but no

TABLE I

ADDISON'S DISEASE

Case	*Age*	*Sex*	*Duration of Disease (years)*	*Duration of Treatment (years)*		*Maximal Histamine Response (mEq. in post-histamine hour)*	*Gastric Biopsy Findings*
				Cortisone	*DOCA*		
1	30	M	14½	7	14	0	Atrophy
2	60	M	9	6½	8	0·2	Chronic gastritis and partial glandular atrophy
3	60	M	12½	7	12	0	Normal
4	38	M	10½	7	10	12·2	Not done
5	40	M	10½	7	10	0 (*p*H 5·5)	Chronic gastritis and partial glandular atrophy
6	53	M	4½	4	0	0	Chronic atrophic gastritis
7	38	M	5	4	4	12·8	Chronic gastritis and partial glandular atrophy
8	17	M	0	0	0	27·5	Normal
9	38	M	½	0	0	0·4	Chronic gastritis and partial glandular atrophy
10	46	F	15	5	13	0	Chronic gastritis and partial glandular atrophy
11	41	F	16	5½	16	5·3	Normal
12	57	F	8	7	7	0	Chronic atrophic gastritis
13	42	F	7	4	6½	3·5	Not done
14	40	F	4	0	0	8·7	Normal

TABLE II

HYPOPITUITARISM

Case	*Age*	*Sex*	*Duration of Disease (years)*	*Duration of Treatment (years)*		*Maximal Histamine Response (mEq. HCl in post-histamine hour)*	*Gastric Biopsy Findings*
				Cortisone	*Thyroid*		
1	55	F	23	5	7	6·1	Normal
2	56	F	3½	3	0	6·2	Failed
3	42	F	13	3	3	9	Normal
4	47	F	16	4	2	3·7	Atrophy
5	50	F	22	7	6½	14·2	Normal
6	63	F	11	8	8	14·4	Normal
7	42	F	19½	5	5	1·8	Normal
8	62	F	20	0	0	0	Partial atrophy
				½	0	0	Not repeated
9	53	F	24	0	0	2·9	Normal
10	45	F	21	0	0	7·5	Atrophy

TABLE III

ACID SECRETORY RESPONSE IN PATIENTS SUFFERING FROM ADDISON'S DISEASE OR HYPOPITUITARISM COMPARED WITH NORMAL SUBJECTS

	Mean Age	*Males*			*Mean Age*	*Females*		
		No. of Patients	*Maximal Histamine Response (mEq. HCl in post-histamine hour)*			*No. of Patients*	*Maximal Histamine Response (mEq. HCl in post-histamine hour)*	
			Range	*Mean*			*Range*	*Mean*
Addison's disease	42	9	0-27·5	5·9	45	5	0-8·7	3·5
Hypopituitarism	—	—	—	—	52	10	0-14·4	6·6
Normals (Card and Sircus, 1960)	—	29	10·1-41·5	22·65	—	28	6·2-34·6	17·2

TABLE IV

STATISTICAL ANALYSIS OF ACID SECRETORY RESPONSE

	Males		*Females*	
	No.	*Mean ± SD*	*No.*	*Mean ± SD*
Normals	29	24·11 ± 1·53	28	17·20 ± 1·36
Addison's disease	9	5·90 ± 3·24	5	3·30 ± 1·65
Hypopituitarism			11	5·98 ± 1·52

TABLE V

STATISTICAL ANALYSIS OF ACID SECRETORY RESPONSE

	Difference	*Degrees of Freedom*	*t*	*p*
Normals—Addison's disease:				
Males	18·21	36	5·56	<·001
Females	13·70	31	4·11	<·001
Normals—hypopituitarism:				
Females	11·22	37	4·70	<·001

inflammatory signs, while in two others (Cases 6 and 12) the appearances were those of chronic atrophic gastritis, *viz.*, chronic inflammatory signs of mild to moderate degree in addition to glandular atrophy. In five patients (Cases 2, 5, 7, 9, and 10) chronic gastritis was present with only slight glandular atrophy; the mucosa in these patients contained body glands and parietal cells were generally conspicuous; the inflammatory signs were mild to moderate except in one patient where they were fairly severe; in two patients (Cases 2 and 4), mitotic figures were conspicuous in the necks of the glands, a feature indicating active regeneration. Intestinal metaplasia was present in only one of the eight patients (Case 6) with an abnormal gastric mucosa. In the remaining four patients, the gastric mucosa had a normal histological structure. Body glands were numerous and regularly arranged and no inflammatory signs were present. It is of considerable interest, however, that one of these patients (Case 3) had achlorhydria.

Hypopituitarism In three patients, the gastric mucosa was abnormal while in the other six patients it was normal.

In two patients (Cases 4 and 10) the histological appearances were those of complete gastric atrophy, the mucosa being thin and containing only glands of pyloric type; parietal cells were absent and there were no inflammatory signs. Intestinal metaplasia was also absent. In another patient (Case 8), there was partial gastric atrophy.

DISCUSSION

This study has confirmed the previous observations of diminished gastric secretion of hydrochloric acid in Addison's disease and hypopituitarism, and it has shown that the high incidence of achlorhydria is frequently associated with atrophic and inflammatory changes in the gastric mucosa, particularly the former. In most cases there was good correlation between the degree of pathological change and the level of acid secretion but there were some exceptions, and it is of particular interest that in one case of Addison's disease (Table 1, Case 3) complete achlorhydria was associated with a normal gastric biopsy. This finding is not entirely unexpected since it is reasonable to assume that gastric secretion is governed not only by size of the parietal cell mass, but also by the reactivity of the parietal cells present and the level of circulating blood hormones.

These functional and structural changes were found more commonly in Addison's disease than in hypopituitarism and this may be a reflection of the relatively greater depression of adrenal function which usually occurs in the former condition. One case only of achlorhydria in hypopituitarism was noted and this persisted after six months' therapy with cortisone. It is, of course, possible that a higher percentage of patients suffering from hypopituitarism in this present investigation would have been achlorhydric had all 10 patients been untreated, but it is significant in this respect that the mean gastric secretion of the whole group was considerably lower than that for control subjects. Spence and Witts (1939) and Kyle (1955), however, have each described a case of hypopituitarism with achlorhydria and gastric atrophy, as judged through a gastroscope in which there was a response to treatment. The first patient was treated with an extract of pig pituitary gland and human urine of pregnancy and the second with cortisone and thyroid extract; in both cases the gastric secretion and gastroscopic appearances returned to normal.

Despite therapy with cortisone for four years or more, six of the 14 patients with Addison's disease in the present series remained achlorhydric. Soffer (1956) related the presence of achlorhydria or hypochlorhydria in Addison's disease to the clinical status of the patient and concluded that the disturbance was one of body fluids and electrolytes which was reversible. It has been shown, moreover, that cortisone administration to normal individuals increases greatly the secretion of hydrochloric acid (Gray, Benson, Spiro, and Reifenstein, 1951); Kirsner, Klotz, and Palmer, 1952) and that there is a diminished excretion of uropepsin in Addison's disease which returns to normal or higher than normal with glucocorticoid replacement therapy (Gray, 1959). Stempien and Dagradi (1954) reported one patient with adrenal insufficiency and normal gastroscopic appearances whose gastric acid secretion was markedly impaired on histamine stimulation which rapidly improved following treatment with cortisone, and Welbourn and Code (1953) demonstrated that prior adrenalectomy of pylorus-ligated rats produced a significant decrease of gastric acid secretion which was restored to normal with cortisone therapy. Such reports have led to the formulation of the 'permissive' theory by Krakauer, Ramsey, and Gray (1955) which suggests that the adrenal corticoid hormones play a permissive role so that in the presence of excess adrenal hormones the stomach or duodenum may be sensitized to respond by secretory hyperactivity and/or ulceration to stimuli which would normally be innocuous.

The failure of cortisone to restore the acid secretion in six of our patients with Addison's disease and one with hypopituitarism may indicate that in humans some factor or factors other than DOCA in Addison's disease and thyroid extract in hypopituitarism is required for full replacement therapy or that the gastric mucosa had undergone irreversible change before treatment began. It should be noted, however, that in Cases 6 and 12 (Table I), where treatment was begun within a matter of months after the development of symptoms, there was no return of gastric acid secretion on maximal histamine stimulation.

We wish to thank Sir Derrick Dunlop for permission to investigate these patients, and we are very grateful to Dr. W. I. Card and Dr. W. Sircus for their generous help in supplying figures of normal gastric secretion levels. Dr. Barnet Woolf gave generous help with the statistical analysis.

REFERENCES

Baker, B. L., and Abrams, G. D. (1954). Effect of hypophysectomy on the cytology of the fundic glands of the stomach and on the secretion of pepsin. *Amer. J. Physiol.*, **177**, 409-412.

——, and Bridgman, R. M. (1954). The histology of the gastrointestinal mucosa (rat) after adrenalectomy or administration of adreno-cortical hormones. *Amer. J. Anat.*, **94**, 363-387.

Card, W. I., and Marks, I. N. (1960). The relationship between the acid output of the stomach following "maximal" histamine stimulation and the parietal cell mass. *Clin. Sci.*, **19**, 147-163.

——, and Sircus, W. (1958). In *Modern Trends in Gastro-enterology*, (*2nd Series*), p. 177. Editor, F. Avery Jones. Butterworth, London.

——,—— (1960). Personal communication.

Crosby, W. H., and Kugler, H. W. (1957). Intraluminal biopsy of the small intestine: the intestinal biopsy capsule. *Amer. J. dig. Dis.*, n.s. **2**, 236-241.

Escamilla, R. F., and Lisser, H. (1942). Simmonds' disease: a clinical study with review of the literature; differentiation from anorexia nervosa by statistical analysis of 595 cases, 101 of which were proved pathologically. *Ibid*, **2**, 65-96.

Feyrter, F., and Klima, R. (1952). Über die Magenveränderungen bei der Addisonschen Krankheit. *Dtsch. med. Wschr.*, **77**, 1173-1175.

Friedman, M. H. F. (1953). The response of different regions of the gastrointestinal tract to normal and abnormal stimuli (influence of feeding inert bulk material and of hypophysectomy). *J. nat. Cancer Inst.*, **13**, 1035-1038.

Grawitz, E. (1907). Über Heilung des Morbus Addisonii nebst Bemerkungen über die Pathogenese dieser Krankheit. *Dtsch. med. Wschr.*, **2**, 1084-1086.

Gray, S. J. (1959). Present status of endocrine influences upon the stomach and their relationship to peptic ulcer disease. *Gastroenterology*, **37**, 412-420.

——, Benson, J. A., Jr., Spiro, H. M., and Reifenstein, R. W. (1951). Effects of ACTH and cortisone upon the stomach: its significance in the normal and in peptic ulcer. *Ibid*, **19**, 658-673.

Haeger, K., Jacobsohn, D., and Kahlson, G. (1953). Atrophy of the gastrointestinal mucosa following hypophysectomy or adrenalectomy *Acta physiol. scand.*, **30**, Suppl. 111, pp. 161-169.

Kay, A. W. (1953). Effect of large doses of histamine on gastric secretion of HCl: an augmented histamine test. *Brit. med. J.*, **2**, 77-80.

Kirsner, J. B., Klotz, A. P., and Palmer, W. L. (1952). Unfavorable course of gastric ulcer during administration of ACTH and cortisone. *Gastroenterology*, **20**, 27-29.

Krakauer ,L. J., Ramsey, C. G., and Gray, S. J. (1955). The relationship of gastric activity and adrenocortical function: a correlative study. *Clin. Res. Proc.*, **3**, 131.

Kyle, J. (1955). Gastric secretion in Simmonds's disease. *Lancet*, **2**, 724-725.

Marañon, G., Sala, P., and Arguelles, G. (1934). Digestive symptoms in chronic suprarenal insufficiency (Addison's disease). *Endocrinology*, **18**, 497-504.

Marks, I. N., and Drysdale, K. M. (1957). A modification of Zimmermann's method for differential staining of gastric mucosa. *Stain Technol.*, **32**, 48.

Rowntree, L. G., and Snell, A. M. (1931). *A Clinical Study of Addison's Disease*. Saunders, Philadelphia and London.

Soffer, L. J. (1956). *Diseases of the Endocrine Glands*, 2nd ed. Lea and Febiger, Philadelphia.

Sorkin, S. Z. (1949). Addison's disease. *Medicine* (*Baltimore*), **28**, 371-425.

Spence, A. W., and Witts, L. J. (1939). Substitution therapy in hypopituitarism. *Quart. J. Med.*, **32**, (n.s. **8**), 69-77.

Stempien, S. J., and Dagradi, A. (1954). The histamine response of the gastric mucosa in a patient with adrenal insufficiency: effect of cortisone administration. *Gastroenterology*, **27**, 358-362.

Welbourn, R. B., and Code, C. F. (1953). Effects of cortisone and of adrenalectomy on secretion of gastric acid and on occurrence of gastric ulceration in the pylorus-ligated rat. *Ibid*, **23**, 356-362.

Gut, 1961, **2**, 168

Studies on the site of fat absorption

2 Fat balances after resection of varying amounts of the small intestine in man

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SYNOPSIS This paper demonstrates that in man, as in the rat, increasing amounts of fat reach more distal levels of the small intestine as the dietary load increases.

Opinions have varied as to the site of fat absorption in the small intestine of experimental animals (Bernard, 1856; Frazer, 1943; Kremen, Linner, and Nelson, 1954; Benson, Chandler, Vansteenhuyse, and Gagnon, 1956; Turner, 1958; Booth, Read, and Jones, 1961). It is now clear that the site where fat is normally absorbed depends on the dietary load. When a small amount of fat is given to a rat, for instance, absorption takes place almost entirely in the jejunum. If larger quantities are fed larger amounts are absorbed in the jejunum, but at the same time an increasing proportion of the ingested fat escapes absorption in the upper intestine and passes on into the ileum where absorption then also occurs (Booth *et al.*, 1961).

The site of absorption of fat in man is uncertain. Borgström, Dahlqvist, Lundh, and Sjövall (1957), using an intestinal intubation technique, have claimed that fat is absorbed in the proximal 100 cm. of the jejunum. This finding, however, conflicts with the clinical observation that steatorrhoea may follow resection of the ileum (Cox, Meynell, Cooke, and Gaddie, 1958; Kalser, Roth, Tumen, and Johnson, 1959). It seemed possible that in man, as in experimental animals, the site of fat absorption might depend on the amount of fat fed. In order to test this hypothesis, we have studied fat balances in a group of patients who had undergone resection of varying amounts of the small intestine. The patients were grouped according to whether the proximal or distal small intestine had been resected, since experimental studies in animals suggest that jejunal resection is less likely to disturb intestinal function than resection of the ileum (Jensenius, 1945; Kremen *et al.*, 1954).

Fat balances were first carried out using diets containing between 30 and 75 g. daily. The results of these balances indicated how much of the small intestine is required to absorb moderate amounts of fat normally. Increasing fat diets were then given to four patients, for if the site of fat absorption is related to the dietary fat, it might be expected that increasing fat diets would cause increasing degrees of steatorrhoea in patients with intestinal resections.

PATIENTS STUDIED

Fat balances were carried out in seven patients who had undergone resection of varying amounts of the distal small intestine (Cases 1 to 7) and two patients who had resections of the proximal intestine (Cases 8 and 9). An approximate estimate of the length of intestine resected in each patient was obtained from the surgical operation specimens and by subsequent barium follow-through examination. Clinical details, including the data from which the extent of the resection in each patient was assessed are given in the Appendix, and the lengths of intestine either resected or assumed to be remaining in the different patients are given in Tables I and II.

MATERIALS AND METHODS

Patients were studied in a metabolic ward. The diets containing from 30 to 100 g. of fat have been described in detail by King and Wootton (1956). Where additional fat was given, it was invariably added in the form of butter fat. The amount of each diet which was rejected was analysed.

ANALYSIS OF FAECAL FAT EXCRETION The methods were those described by King and Wootton (1956). After a preliminary equilibration period of three days on each diet, the stools were collected for two consecutive three-day periods into suitable metal containers. The specimens were first weighed and homogenized and a sample of this

homogenate was then dried. This dried aliquot was treated with hydrochloric acid and extracted with ether, the extracted fat being determined gravimetrically. The mean faecal fat excretion during each of the three-day periods was then calculated. Using this technique, control subjects usually excrete less than 6 g. of fat daily when taking diets containing between 50 and 100 g. of fat.

The difference between the dietary fat and the fat excreted was assumed to be the amount absorbed. While this calculation does not take into account the small amount of fat in the faeces that may be of endogenous origin, it gives a reasonable approximation of fat absorption.

RESULTS

MODERATE DIETARY INTAKES The dietary intakes, faecal fat excretion, and estimated fat absorption from diets containing moderate amounts of fat (between 30 and 75 g. daily) are shown in Tables I (distal resections) and II (proximal resections).

Distal resections The results of the fat balances in the seven patients who had undergone resection of varying amounts of the distal small intestine (Cases 1 to 7) are given in Table I and illustrated in Fig. 1.

Two patients (Cases 1 and 2) had no significant steatorrhoea (Fig. 1); these two patients had lost the ileocaecal valve together with only 6 or 8 ft. of the distal ileum.

TABLE I

FAT BALANCES DURING TWO CONSECUTIVE THREE-DAY PERIODS AFTER RESECTION OF DISTAL SMALL INTESTINE

Case No.	*Amount of Intestine Resected*	*Dietary Fat (g. per day)*		*Faecal Fat (g. per day)*		*Fat Absorbed (g. per day)*	
		1	*2*	*1*	*2*	*1*	*2*
1	6 ft. of terminal ileum	47	49	6·2	4·6	41·8	44·4
2	8 ft. of terminal ileum	64	63	6·1	6·2	57·9	56·8
		98	98	14·0	12·0	84·0	86·0
		150	150	15·0	15·0	135·0	135·0
3	10 ft. of ileum	73	71	9·7	9·5	63·4	61·5
4	Ileum and all but proximal 6 ft. of jejunum	29	31	9·0	11·0	20·0	20·0
		51	54	11·0	15·0	40·0	39·0
		98	98	25·0	18·0	73·0	80·0
5	Ileum and all but proximal 6 ft. of jejunum	47	50	13·0	22·0	34·0	28·0
		98	98	26·0	30·0	72·0	68·0
6	Ileum and all but proximal 4 ft. of jejunum	63	62	23·0	19·0	40·0	43·0
7	Ileum and all but proximal 8 in. of jejunum	30	—	15·0	—	15·0	—

The patients with larger distal resections had steatorrhoea and its degree depended on the extent of their resection. One patient (Case 3), who had undergone resection of approximately 10 ft. of the ileum had mild steatorrhoea, excreting 9·5 and 9·7 g. fat daily during successive balance periods (Fig. 1). Two other patients (Cases 4 and 5), both of whom

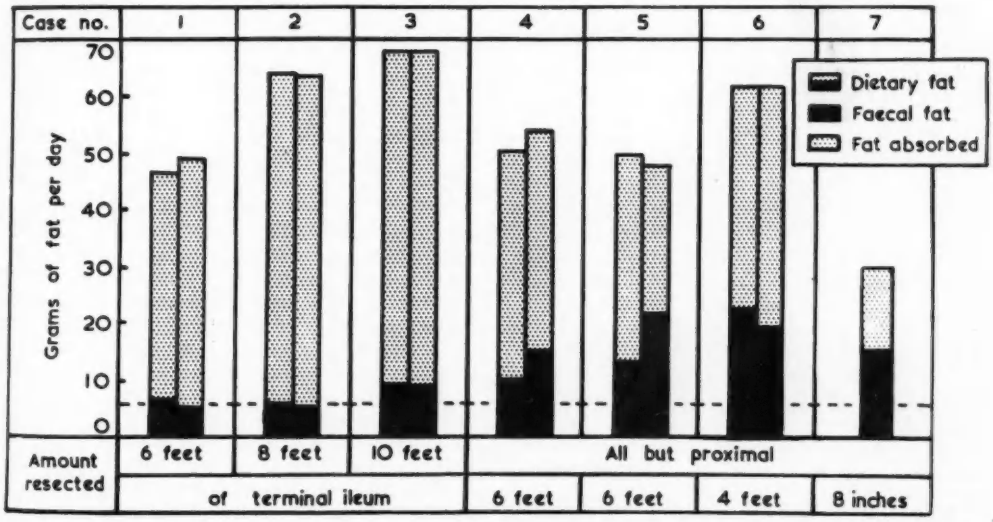

FIG. 1. *Dietary fat, faecal fat excretion, and fat absorption (g. per day) during successive three-day balance periods in patients who had undergone resection of varying amounts of the distal small intestine (Cases 1 to 7). The interrupted line indicates the upper limit of normal faecal fat excretion.*

had more extensive distal resections, had more marked steatorrhoea; these patients excreted between 11·0 and 22·0 g. of fat daily.

However, even though there might be severe steatorrhoea after a large distal resection, the proximal jejunum was capable of absorbing a considerable proportion of the dietary fat. Although only 4 ft. of the proximal jejunum remained in Case 6, for instance, more than 40 g. of fat was absorbed by this patient from a daily intake of 62 g. (Case 6, Fig. 1, Table I). Furthermore, another patient (Case 7), who had only the duodenum and proximal 8 in. of the jejunum remaining, absorbed as much as 15 g. of fat from a daily intake of 30 g. (Fig. 1, Table I).

Proximal resections The results of similar balances in the two patients who had undergone resection of the proximal small intestine are given in Table II and illustrated in Fig. 2.

The first of these patients (Case 8) had lost the proximal 8 ft. of the jejunum; the daily faecal fat excretion was 6·2 and 6·8 g. during two successive balance periods on diets containing 48·9 and 46·7 g. of fat (Fig. 2, Table II), indicating that most of the dietary fat was absorbed (Table II).

The resection in the second patient was very much more extensive, only 15 in. of the terminal ileum remaining. This patient had moderate steatorrhoea, excreting 12·1 and 13·4 g. of fat daily, but he absorbed more than 35 g. of fat from the short segment of remaining distal small intestine.

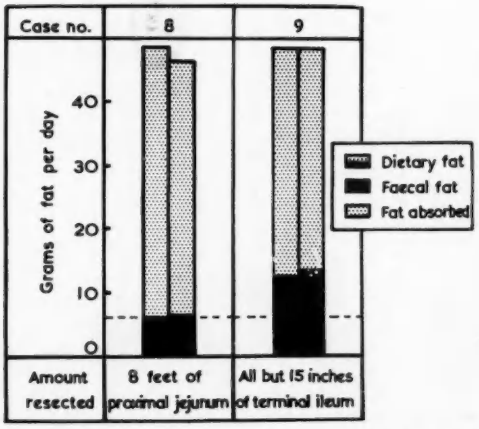

FIG. 2. *Dietary fat, faecal fat excretion, and fat absorption (g. per day) during successive balance periods in patients who had undergone resection of the proximal small intestine (Cases 8 and 9). The interrupted line indicates the upper limit of normal faecal fat excretion.*

TABLE II

FAT BALANCES DURING TWO CONSECUTIVE THREE-DAY PERIODS AFTER RESECTION OF PROXIMAL SMALL INTESTINE

Case No.	Amount of Intestine Resected	Dietary Fat (g. per day)		Faecal Fat (g. per day)		Fat Absorbed (g. per day)	
		1	2	1	2	1	2
8	8 ft. of proximal jejunum	48·9	46·7	6·2	6·8	42·7	39·9
		98·3	98·0	6·9	7·0	91·4	91·0
		150·0	152·0	5·0	11·4	145·0	141·6
9[1]	All jejunum and all but distal 15 in. of terminal ileum	48·5	48·5	12·1	13·4	36·4	35·1

[1]Balances were carried out during two consecutive four-day periods in this patient.

EFFECT OF INCREASING DIETARY FAT The results of the balances when increasing fat diets were given are also shown in Tables I and II.

Distal resections Increasing fat diets (between 30 and 150 g. daily) were given to three of the patients who had undergone distal resections (Cases 2, 4, and 5). The results of the two successive three-day balance periods on each diet are given in Table I. The mean faecal fat excretion during the total six-day period of each diet is illustrated in Fig. 3, together with the corresponding amount of fat absorbed.

One of these patients (Case 2) had lost only the terminal 8 ft. of the ileum and had no steatorrhoea when taking a diet containing 63 g. of fat daily. However, when the dietary fat was increased to 98 or 150 g. daily, the mean daily faecal fat excretion rose to 13 and 16 g. respectively (Fig. 3, Table I). Although this patient developed steatorrhoea when taking the higher fat diets, the actual amount of fat absorbed increased as the dietary fat was increased (Fig. 3).

The two other patients (Cases 4 and 5) had approximately 6 ft. of the proximal jejunum remaining and both had steatorrhoea when receiving diets containing 50 g. of fat (Fig. 1). One of these patients (Case 4, Fig. 3) was given diets containing 30, 50, and 100 g. of fat daily. As the dietary fat was increased, there was a progressive increase in faecal fat excretion but at the same time the amount of fat absorbed from the remnant of proximal small intestine also increased (Fig. 3, Table I). Similarly in the other patient (Case 5, Fig. 3) there was an increase in both faecal fat excretion and fat absorption when the dietary fat was increased from 50 to 100 g. daily (Fig. 3, Table I).

Proximal resections Increasing fat diets (from 50 to 150 g. daily) were also given to the patient who had undergone resection of 8 ft. of the proximal

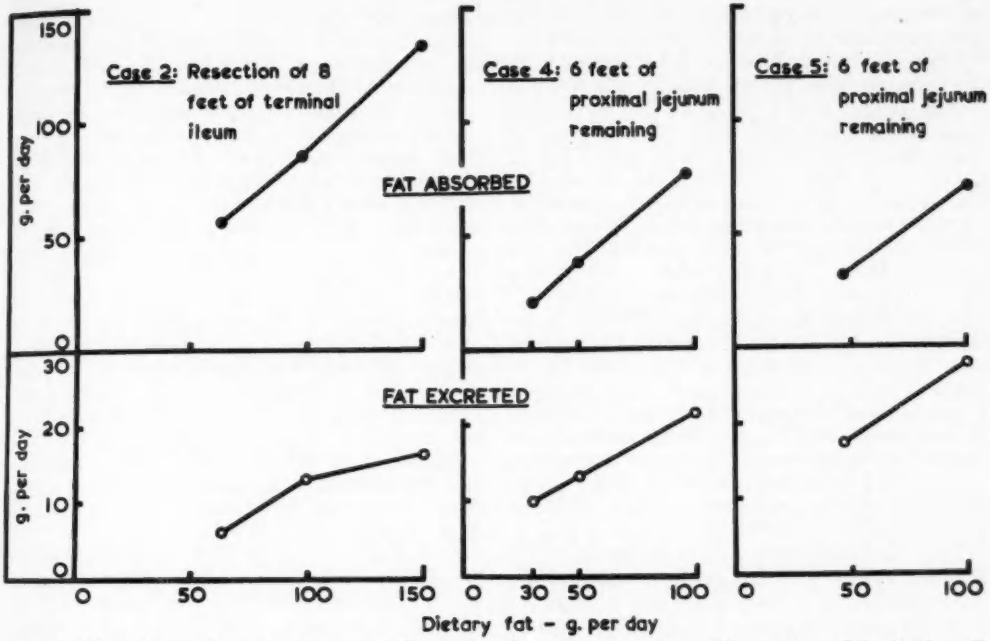

FIG. 3. *Effect of increasing fat in diets on fat absorption and excretion in patients with resections of the distal small intestine (Cases 2, 4, and 5).*

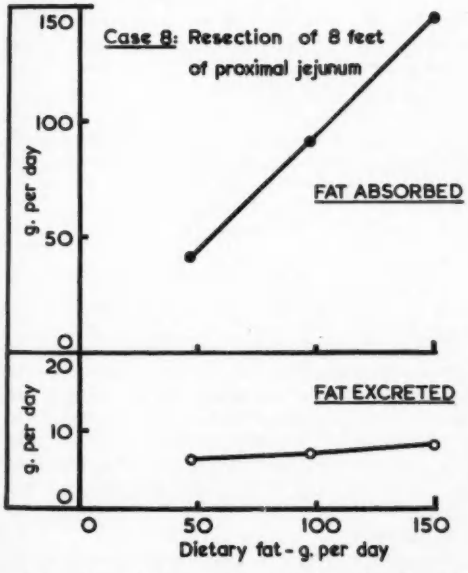

FIG. 4. *Effect of increasing fat in diets on fat absorption and excretion.*

jejunum. The results of the balances are given in Table II and illustrated in Fig. 4.

This patient excreted a mean of only 6·5 g. of fat daily during the six-day balance when he received a diet containing just less than 50 g. of fat (Fig. 1). When the dietary fat was increased, there was a slight increase in faecal fat excretion (Fig. 4) but the mean amount of fat excreted daily when the diet contained as much as 150 g. of fat was only 8·2 g. As shown in Fig. 4, the amount of fat absorbed by this patient increased progressively as the dietary fat was increased (Table II).

DISCUSSION

The results in the patients subjected to distal resections (Cases 1 to 7, Fig. 1) show that in man the jejunum can absorb fat well. They also indicate that although the proximal ileum may be required for completing the absorption of fat (Case 3, Fig. 1), the distal 6 to 8 ft. do not appear to be necessary when moderate diets are given, even when the ileocaecal valve has been resected (Cases 1 and 2, Fig. 1).

The effects of increasing the dietary fat in three of these patients suggest that the small intestine of

man responds to an increased dietary load in the same way as that of the rat (Booth *et al.*, 1961), for the jejunum absorbed more fat when the dietary fat was increased. Two of these patients (Cases 4 and 5, Fig. 3) had only 6 ft. of the proximal small intestine remaining, and there was a progressive rise in fat absorption with increase in the dietary intake (Fig. 3, Table I). However, the increased fat absorption in these patients was only achieved at the expense of an increased faecal fat excretion (Cases 4 and 5, Fig. 3). This suggests that, as also occurs in the rat, increasing amounts of fat may reach more distal regions of the small intestine when the dietary fat is increased. The results in the third patient (Case 2) are in keeping with this concept. This patient had lost only the terminal 8 ft. of the ileum and her remaining intestine was capable of absorbing normal amounts of fat from a moderate dietary intake (Fig. 1). However, when greater amounts of fat were given, the faecal fat excretion increased and steatorrhoea developed (Fig. 3), suggesting that some fat may reach the distal ileum and be absorbed there when the dietary intake is large.

Like the upper jejunum, the terminal ileum was shown to absorb fat well. Case 9, for instance, had only the distal 15 in. of the ileum remaining, yet he absorbed more than 35 g. of fat from this remnant of the distal small intestine (Fig. 2). Similarly, Case 8, who had lost the proximal 8 ft. of the jejunum, absorbed fat almost normally when given a moderate dietary intake (Fig. 2). In this patient, increasing the dietary fat resulted in a progressive increase in absorption and caused only a slight increase in the faecal fat excretion (Fig. 4); this contrasts with the steatorrhoea produced by similar diets in the patient with a comparable distal resection (Case 2, Fig. 3).

Repeated studies have demonstrated that when normal persons are given diets containing between 30 and 350 g. of fat daily, there is a slight increase in the faecal fat excretion, which may reach as much as 10 g. a day on the highest dietary intakes (Wollaeger, Comfort, and Osterberg, 1947; Annegers Boutwell, and Ivy, 1948), but in terms of the amount of fat absorbed there is a progressive increase in fat absorption as the dietary intake is increased. Masterton, Lewis, and Widdowson (1957) found that two polar explorers were able to absorb 263 and 276 g. of fat respectively from diets containing 273 and 285 g. of fat daily, diets which were designed to meet their high energy requirements. The results reported in this paper provide a rational explanation for this apparently unlimited capacity of the human small intestine to absorb fat. When a moderate dietary intake is given, it seems likely that fat absorption occurs predominantly in the jejunum (Fig. 1). As the dietary load is increased, the amount of fat absorbed by the jejunum increases (Fig. 3). At the same time increasing amounts of fat escape absorption in the jejunum (Fig. 3) and presumably pass on into the ileum, where absorption may then normally occur, for, as shown by the results in Case 8, the distal intestine is also capable of absorbing increasing amounts of fat when presented with an increased load and very little fat passes through it unabsorbed (Fig. 4). The ileum therefore appears to become progressively more important as a site of fat absorption when the dietary fat is increased.

Since both jejunum and ileum are capable of absorbing fat well, and since both respond to an increased dietary load with an increased absorption (Figs. 3 and 4), it is not surprising that in man the amount of fat which can be absorbed is almost unlimited. Fat absorption must therefore be controlled by the appetite of the individual and not by any intramucosal mechanism such as that which regulates the absorption of vitamin B_{12} (Glass, Boyd, and Stephanson, 1954; Swendseid, Gasster, and Halsted, 1954; Booth, 1958).

In conclusion, it appears that in man, as in the rat, the jejunum is the major area of fat absorption. As far as fat is concerned, the ileum seems to represent the main reserve of the small intestine which, like other essential organs such as the liver and kidney, is equipped to deal with far more than basic metabolic requirements. Although the ileum may be of relatively minor importance when moderate amounts of fat are taken, the results given in this paper suggest that it is likely to be of greater significance to those who enjoy gargantuan appetites. For individuals such as polar explorers who must take a high fat diet to meet high caloric requirements (Masterton *et al.*, 1957), a functioning ileum is probably essential.

SUMMARY

Fat balances were carried out in a group of patients who had undergone resection of varying amounts of the small intestine.

When moderate fat diets were given, resection of 6 or 8 ft. of the ileum did not interfere with fat absorption. Resection of larger amounts of the distal intestine caused steatorrhoea but a considerable proportion of the dietary fat was absorbed when only 4 to 6 ft. of the proximal intestine remained and some was absorbed even when all but the duodenum and proximal 8 in. of the jejunum had been resected.

The results of giving increasing amounts of fat in the diet to patients with resection of the distal small intestine indicate that the jejunum responds

to an increased dietary load with an increased absorption, but increasing amounts of fat escape absorption and are excreted in the faeces when the dietary fat is increased in such patients. These observations suggest that in man, as in the rat, increasing amounts of fat reach more distal levels in the small intestine as the dietary load increases. It seems likely that the jejunum in man is the major site of fat absorption when moderate diets are given, but the ileum probably becomes progressively more important with increase in the dietary fat.

Fat was absorbed well by a patient with a resection of 8 ft. of the proximal jejunum even when diets of up to 150 g. of fat were given. The terminal ileum can absorb fat, for a patient with only 15 in. of the terminal ileum remaining absorbed more than 35 g. from a diet containing 48 g. of fat.

We wish to thank Dr. F. Avery Jones for permission to carry out studies in Case 8, and Dr. Barbara Clayton for her kindness in allowing us to quote the results in Case 9. We also thank the physicians and surgeons of Hammersmith Hospital for their cooperation.

APPENDIX

Clinical details and results of other intestinal function tests in many of these patients were given at the International Congress of Gastroenterology at Leyden, 1960 (Booth and Mollin, 1961).

DISTAL RESECTIONS

CASE 1 A 30-year-old man developed gangrene of the terminal ileum as a result of a volvulus. Approximately 6 ft. of the terminal ileum was resected (surgeon's estimate), the ileum being anastomosed to the caecum. A barium follow-through demonstrated a normal pattern of the small intestine but very rapid transit, barium reaching the caecum within 15 minutes.

CASE 2 A 36-year-old woman had intermittent diarrhoea, passing two to three watery motions daily. Eight years before, laparotomy had revealed Crohn's disease of the terminal ileum and approximately 4 ft. of the terminal ileum and the ascending colon were removed. A recurrence two years later necessitated resection of a further 3 to 4 ft. of terminal ileum, end-to-end ileo-transverse anastomosis being performed. A recent laparotomy for an obstruction due to an adhesion had shown a well-functioning ileo-transverse anastomosis and no evidence of Crohn's disease. A barium follow-through revealed a normal small bowel pattern, barium passing into the colon in two hours.

CASE 3 A 40-year-old man had a 20-year history of tuberculosis involving first the right ankle joint and then the terminal ileum and caecum. Approximately 10 ft. of the distal intestine had subsequently been removed (surgeon's estimate), the small intestine being anastomosed to the transverse colon by a side-to-side anastomosis. A barium follow-through revealed a normal pattern of the residual small intestine and the barium passed into the colon in two hours. There appeared to be a small blind end of the terminal ileum but there was no stasis within this loop.

CASE 4 A young woman aged 28 developed extensive gangrene of the distal small intestine caused by a volvulus. The affected part of the gut was resected, the surgeon estimating that approximately 6 ft. of the proximal intestine remained. End-to-side jejunocaecal anastomosis was performed. A subsequent barium follow-through revealed a normal pattern of the remaining small intestine, and barium reached the caecum within 20 minutes.

CASE 5 A 30-year-old woman had first developed Crohn's disease of the terminal ileum associated with a peri-rectal abscess 10 years ago. Resection of the distal small intestine had been carried out on four occasions. At her last operation, two years before, blind loops of the distal intestine were resected and the jejunum was anastomosed end-to-end to the transverse colon. The surgeon estimated that approximately 6 ft. of the jejunum remained. There was no evidence of Crohn's disease at this time. A barium follow-through revealed a normal pattern of the residual small bowel but the barium had passed into the colon by three quarters of a hour.

CASE 6 An imbecile hospital patient aged 24 years had developed a volvulus of the small intestine causing extensive gangrene two months before. The right side of the colon, together with all but the proximal 3 to 4 ft. of the jejunum (surgeon's estimate) were resected, end-to-end anastomosis being performed between the upper jejunum and the transverse colon. A barium follow-through was hampered by some delay in gastric emptying, but the pattern of the remaining small intestine was normal and barium passed into the colon within one and a half hours.

CASE 7 An 80-year-old widow had developed a thrombosis of the superior mesenteric artery causing extensive gangrene of the small intestine. The ileum and all but the proximal 8 in. of the jejunum were resected, but jejunal remnant being anastomosed end-to-end to the ascending colon. A barium follow-through revealed slight dilatation of the residual small intestine; some of the barium had reached the colon within five minutes. (This case has been reported in detail by Harrison and Booth, 1960.)

PROXIMAL RESECTIONS

CASE 8 A 71-year-old man had extensive jejunal diverticulosis requiring resection of 8 ft. of the proximal jejunum, the operation specimen being measured after the operation. End-to-end anastomosis was performed. A barium follow-through two years later revealed slight dilatation of the bowel immediately distal to the anasto-

mosis but no other abnormality. Barium had passed into the colon within four hours.

CASE 9 An 18-month-old child had undergone three successive operations on the small intestine for ileo-ileal intussusception. Extensive resections had been carried out, the stomach eventually being anastomosed to the terminal ileum. The amount of ileum remaining was estimated to be 15 in. (surgeon's estimate). A barium follow-through eight months later revealed slight dilatation of the remaining small bowel; barium reached the colon in one and three quarter hours. (This case has been reported in detail by Clayton and Cotton, 1961.)

REFERENCES

Annegers, J. H., Boutwell, J. H., and Ivy, A. C. (1948). The effect of dietary fat on faecal fat excretion and subjective symptoms in man. *Gastroenterology*, **10**, 486-495.

Benson, J. A. Jr., Chandler, G. N., Vansteenhuyse, F. E., and Gagnon, J. O. (1956). Studies concerning the site of fat absorption in the small intestine of the rat. *Ibid*, **30**, 53-61.

Bernard, C. (1856). *Mémoire sur le pancréas et sur la role du suc pancréatique dans les phénomènes digestifs, particulièrement dans la digestion des matières grasses neutres*. Baillière, Paris.

Booth, C. C. (1958). *An Experimental and Clinical Study of the Intestinal Absorption of Vitamin B_{12}*. M.D. Thesis, University of St. Andrews.

——, and Mollin, D. L. (1961). The effect of resection of the small intestine in man. *Proc. Internat. Conf. Gastroenterol.*, Leyden 1960.

——, Read, A. E., and Jones, E. (1961) Studies on the site of fat absorption. 1. The sites of absorption of increasing doses of ^{131}I-labelled triolein in the rat. *Gut*, **2**, 23.

Borgström, B., Dahlqvist, A., Lundh, G., and Sjövall, J. (1957). Studies of intestinal digestion and absorption in the human. *J. clin. Invest.*, **36**, 1521-1536.

Clayton, B. E., and Cotton, D. A. (1961). A study of malabsorption following resection of the entire jejunum and the proximal half of the ileum. *Gut*, **2**, 18.

Cox, E. V., Meynell, M. J., Cooke, W. T., and Gaddie, R. (1958). The folic acid excretion test in the steatorrhea syndrome. *Gastroenterology*, **35**, 390-397.

Frazer, A. C. (1943). Differentiation in the absorption of olive oil and oleic acid in the rat. *J. Physiol. (Lond.)*, **102**, 306-312.

Glass, G. B. J., Boyd, L. J., and Stephanson, L. (1954). Intestinal absorption of vitamin B_{12} in man. *Science*, **120**, 74-75.

Harrison, R. J., and Booth, C. C. (1960). Massive resection of the small intestine after occlusion of the superior mesenteric artery. *Gut*, **1**, 237-241.

Jensenius, H. (1945). Results of experimental resections of the small intestine on dogs. Nyt nordisk Forlag, Copenhagen.

Kalser, M. H., Roth, J. L. A., Tumen, H., and Johnson, T. A. (1959). Relation of Small Bowel Resection to Nutrition in Man. In *Proceedings of the World Congress of Gastroenterology, Washington* 1958. Vol. I, pp. 458-468. Williams and Wilkins Co., Baltimore.

King, E. J., and Wootton, I. D. P. (1956). *Micro-analysis in Medical Biochemistry*. 3rd ed. Churchill, London.

Kremen, A. J., Linner, J. H., and Nelson, C. H. (1954). An experimental evaluation of the nutritional importance of proximal and distal small intestine. *Ann. Surg.*, **140**, 439-447.

Masterton, J. P., Lewis, H. E., and Widdowson, E. M. (1957). Food intakes, energy expenditures, and faecal excretions of men on a polar expedition. *Brit. J. Nutr.*, **11**, 346-358.

Swendseid, M. E., Gasster, M., and Halsted, J. A. (1954). Limits of absorption of orally administered vitamin B_{12}; effect of intrinsic factor sources. *Proc. Soc. exp. Biol. (N.Y.)*, **86**, 834-836.

Turner, D. A. (1958). The absorption, transport and deposition of of fat. *Amer. J. dig. Dis.*, n.s. **3**, (Part 1) 594-640, (Part 2) 682-708.

Wollaeger, E. E., Comfort, M. W., and Osterberg, A. E. (1947). Total solids, fat and nitrogen in the faeces: III. A study of normal persons taking a test diet containing a moderate amount of fat; comparison with results obtained with normal persons taking a test diet containing a large amount of fat. *Gastroenterology*, **9**, 272-283.

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Methods and techniques

The motility of the pelvic colon

1 Motility in normals and in patients with asymptomatic duodenal ulcer

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The study of the motility of the alimentary tract has lagged behind the study of its secretory activity, largely due to difficulties of technique. For many years, radiology has contributed extensively to our knowledge but has obvious limitations. It is now generally agreed that only some form of continuous manometry is suitable for accurate quantitative work. Early studies using large balloons and relatively insensitive manometers are suspect because of the physiological and mechanical artefacts produced by large distending balloons (Quigley and Brody, 1950) although some information about colonic motility has been obtained by this method (Adler, Atkinson, and Ivy, 1941; Spriggs, Code, Bargen, Curtiss, and Hightower, 1951).

More recently, however, the development of transducers, which are small enough to be swallowed, open-ended tubes, and miniature balloons has made it possible to record the unstimulated activity of the alimentary tract. These methods have been applied extensively and successfully to the study of oesophageal motility but very few studies of intestinal motility have been made. A detailed study of the colonic motility of children using open-ended tubes has been presented by Davidson, Sleisenger, Almy, and Levine (1956). This study presents an analysis of the unstimulated motility of the sigmoid colon recorded under resting physiological conditions in adult normal subjects and patients with duodenal ulceration. These later were studied because standard textbooks (Bockus, 1944; Avery Jones and Gummer, 1960) indicate that a number of patients with duodenal ulceration have symptoms of colonic type, and so far no study of colonic motility has been attempted in these patients. It should be emphazised that these patients were symptom free at the time although an ulcer was present.

METHODS

TECHNIQUE OF RECORDING PRESSURE Intraluminal pressures have been recorded using miniature balloons (7 mm. in diameter × 10 mm. length) (Atkinson, Edwards, Honour, and Rowlands, 1957) connected by fine polyethylene tubing to a metal capsule optical manometer of high sensitivity (Rowlands, Honour, Edwards, and Corbett, 1953). The total volume of the recording system is about 1 c.cm. and the change of volume with variation in pressure is very small. Simultaneous recordings from a miniature balloon and an open-ended tube in the same segment of gut are identical except when the balloon is compressed by the muscular wall as at the cardia. Under these conditions the pressures indicated by the balloon are greater than would be recorded from an open-ended tube at the same site (Edwards and Rowlands, 1960). In the sigmoid, however, records from a miniature balloon and open-ended tube are usually identical (Fig. 1). A model of the conditions in the colon was prepared by placing an open-ended tube and a miniature balloon in the same position inside a large soft rubber balloon. The pressure inside the large balloon was varied over a wide range and resulted in similar pressures in the two recording systems. The pressure recorded by the open-ended tube was 13% higher than that recorded by the miniature balloon, but when the latter was directly compressed by the wall of the large balloon it recorded pressures up to 33% higher than the open-ended tube under similar conditions. In addition records from miniature balloons have been compared with those from radio pills, recording from exactly the same position in the gut. An exact qualitative comparison has been obtained although there are some minor quantitative differences (Connell and Rowlands, 1960). Some of the earlier studies reported in this paper were made with open-ended tubes but miniature balloons were preferred subsequently. The open-ended tubes did not consistently give reliable results due to blocking with faeces, even if a slow stream of air under high pressure was bled continuously through them in an effort to keep them patent.

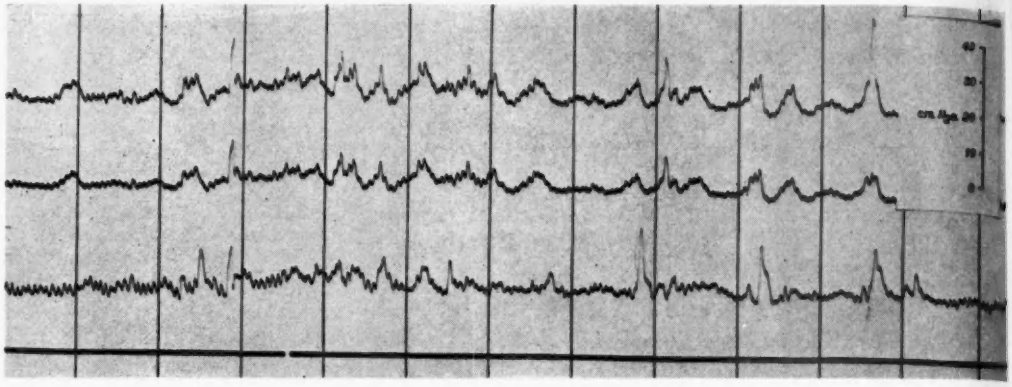

FIG. 1. *Comparison of the records produced by a miniature balloon and an open-ended tube in the same segment of bowel. Top tracing: Miniature balloon. Middle tracing: open-ended tube in same segment. Lower tracing: recording from a different segment of bowel. Verticals = 1 minute intervals. Calibration in 10 cm. water steps.*

In most of the studies, three balloons were used and tied to tubes at distances of 5 cm. from each other.

Sleeves of radio-opaque tubing were interposed between the balloons and a sleeve of fine semi-rigid polyethylene tubing was placed over the connecting tubes to simplify insertion into the colon. The recording tube was connected to the optical manometer and the system adjusted for temperature and pressure so that, under resting conditions, the air in the balloon was at atmospheric pressure when at body temperature. Under these conditions the walls of the balloon were lax. No air was inserted into the balloons.

PATIENTS AND PROCEDURE Colonic motility has been studied in 32 subjects, most of whom were hospital in-patients. Fourteen had radiological and subsequent operative evidence of chronic duodenal ulceration but had no ulcer symptoms during the period of study. The remaining 18 subjects were either normal or suffering from general medical or minor surgical complaints (pilonidal sinus, post-infective fistula-in-ano, intermittent claudication, bleeding haemorrhoids, etc). None had suffered from any prolonged bouts of abdominal pain or any prolonged disorder of bowel function. All were symptom free at the times of study.

In addition, records have been obtained on nine different occasions from two other patients who complained of abdominal pain and in whom repeated investigations over a period of years had been negative.

All studies were made with the patient relaxing on a couch in a quiet room. The subjects were encouraged to read light literature. No food was allowed for two hours before the beginning of the study.

Without any prior preparation of the patient, a small bore Lloyd Davies sigmoidoscope was passed to beyond the rectosigmoid angle without distending the colon with air. The tubes and recording balloons, still attached to the manometer, were passed into the colon through the sigmoidoscope which was then withdrawn. As it is not consistently possible to insinuate the tubes for more than a few centimetres along the colon without tedious and time-consuming manoeuvres, the recording tips were placed routinely at 25, 20, and 15 cm. from the anus. The position of the tube has been checked radiologically in several patients using an image intensifier and it has been shown that its position does not alter appreciably even in studies lasting several hours. After placing the tube in the colon half an hour was allowed to elapse before the recording began and this was continued for periods of up to five hours.

It is essential to record the respiratory and body movement at the same time as colonic motility. This was done by using a piece of gas mask tubing as a stethograph around the upper abdomen and attached to a capsule of the manometer. Without the use of a stethograph no reliable information can be obtained about the faster components of colonic activity.

ANALYSIS OF RECORDS Preliminary observation of the records indicated that when colonic activity was phasic the cycle was nearly always less than 30 minutes in duration although very occasionally periods of inactivity lasting more than one hour were seen. In two patients in whom recordings were made over a period of several hours, analyses were made over consecutive half-hour periods. These indicated that with the exception of the increase of activity associated with eating, the activity of these half-hour periods remained relatively constant (Fig. 2). Eighteen records were analysed over half-hour periods and subsequently over one-hour periods but no significant differences were found in the mean results of the two groups (Table I). Half hour periods of analysis therefore are adequate for studies comparing groups of patients.

Each record was carefully checked against the stethograph and all variations which appeared to represent

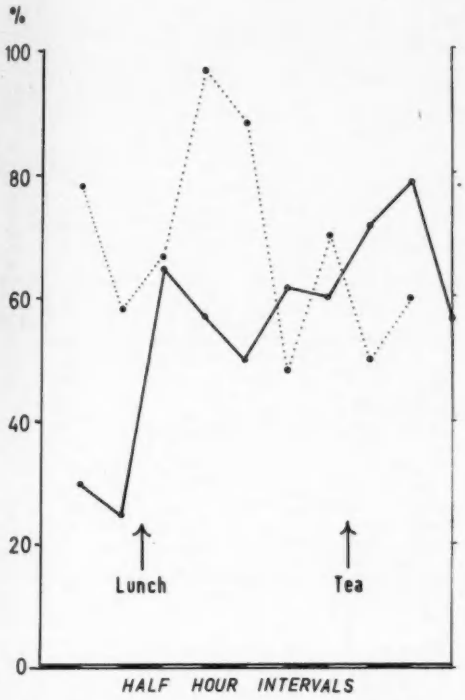

FIG. 2. *The percentage duration of activity of two subjects estimated each half hour on records lasting several hours.*

cardiovascular, respiratory, or somatic movement were excluded from further consideration. The following parameters were measured over a period of 30 minutes in each record:

The duration of colonic activity was measured and expressed as a percentage of the time of analysis. For this purpose activity was defined as any rise in basal pressure which was not accounted for by cardiovascular, respiratory, or somatic movement. For example, in Fig. 3 the base line alterations of the upper two traces would be considered as activity whereas in the bottom trace the only base line alterations can be accounted for as respiratory swings. However, prolonged slow base line alterations lasting many minutes and not amounting to more than 3 or 4 cm. of water pressure have not been considered as activity for the purposes of this analysis.

An arbitary distinction has been made between pressure waves occurring at a frequency of five per minute or more and those at less than five per minute. The amplitude of the slow waves (frequency less than five per minute) was measured and the mean amplitude in each record calculated. In addition, the proportion of the slow waves which represent pressures of more than 20 cm. water was estimated. Where the waves occurred in rhythmic sequence the frequency of this phasic activity was recorded.

An assessment of colonic activity is given by the product of the total duration of activity and the mean amplitude of the slow waves. This figure approximates to that which would be obtained by integrating the area under the trace with a planimeter or by electronic integration (Fig. 4).

The proportion of the time of analysis in which fast waves (five per minute or more) occurred has been recorded.

TABLE I

COMPARISON OF ONE HALF HOUR AND ONE HOUR PERIODS OF ANALYSIS

Subject	*First Half Hour*			*One Hour*		
	Percentage Duration of Activity	*Percentage Waves > 20 cm. Water Pressure*	*Percentage Duration of time in which Fast Waves Occur*	*Percentage Duration of Activity*	*Percentage Waves > 20 cm. Water Pressure*	*Percentage Duration of time in which Fast Waves Occur*
1	49	33	0	29	17	0
2	47	40	2	52	50	1
3	70	33	0	74	32	0
4	23	33	0	25	28	0
5	20	33	0	16	33	0
6	48	12	3	61	16	2
7	0	0	0	0	0	0
8	30	10	0	39	8	0
9	67	0	0	65	0	0
10	72	16	0	69	21	0
11	83	3	5	78	2	6
12	50	2	20	57	1	25
13	47	77	2	43	62	3
14	72	8	0	68	7	0
15	18	0	0	23	0	0
16	73	21	0	60	19	0
17	70	16	0	65	17	0
18	65	8	0	62	8	0
Mean	= 50·2	19·2	1·8	49·2	17·8	2·1
S.D.	= ±23·0	±19·1	±4·6	±21·8	±17·2	±5·8

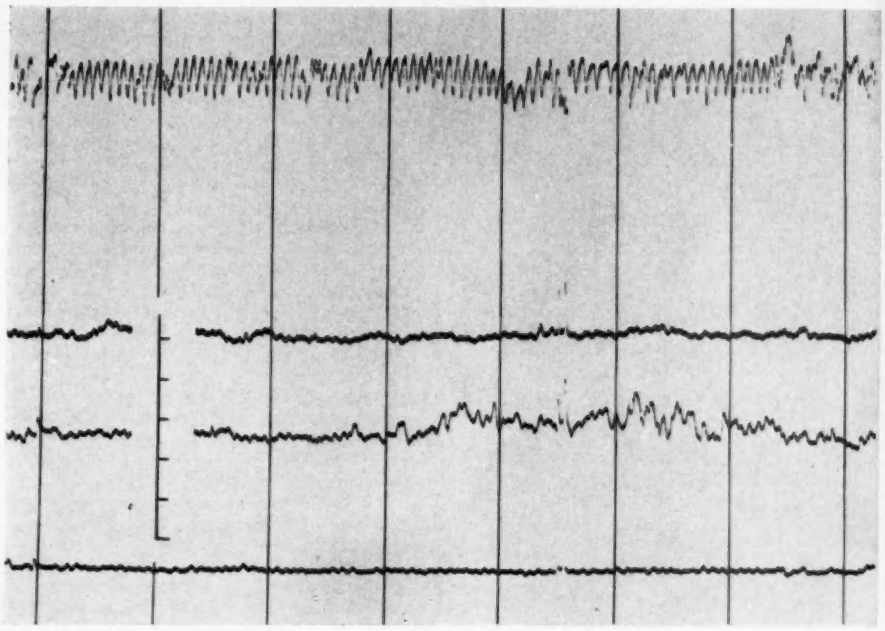

FIG. 3. *Normal colonic activity. The lowest tracing shows only respiratory activity but activity from the colon is present in the other two tracings. Top trace = stethograph. Verticals = 1 minute intervals. Calibration in 10 cm. water steps.*

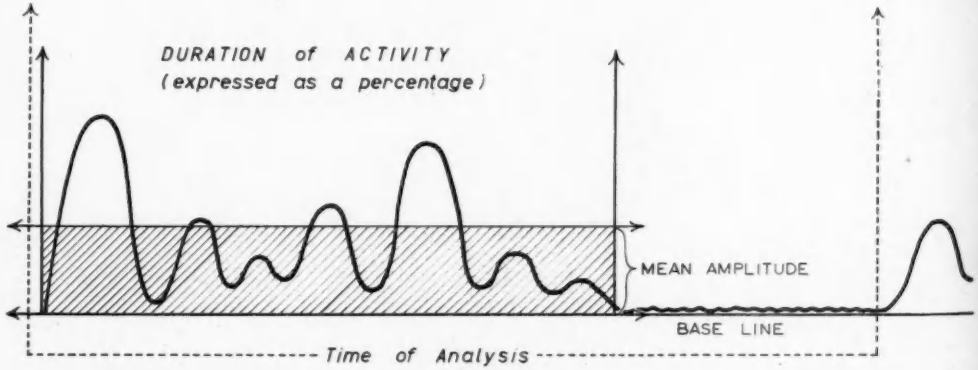

FIG. 4. *Diagrammatic representation of the significance of 'total activity'. Total activity = product of duration of activity (%) × mean amplitude.*

ERROR OF ANALYSIS Analysis of complex records of the type obtained in this study involves an observer error. However, it soon became evident that this error was only significant in estimating the duration of activity. This error has been estimated by determining the duration of activity of each of six records on 10 separate occasions. The results of this analysis are set out in Table IIa. It will be seen that the variation of the results about the mean was approximately the same in the different subjects and not related to the absolute duration of activity. The mean

TABLE II

ERROR OF ANALYSIS

TEN SEPARATE ANALYSES OVER THE SAME 30-MINUTE PERIOD IN SIX DIFFERENT RECORDS

Subject	*Observation*										
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*Mean*	*S.D.*
(a) *Single analysis of the percentage duration of activity*												
1	47	50	45	48	46	57	46	47	43	50	48	±3·9
2	45	47	47	47	43	47	55	42	38	50	46	±4·6
3	17	17	15	23	15	23	15	17	18	18	18	±2·9
4	20	23	15	20	17	20	20	18	13	15	18	±3·0
5	60	60	57	57	58	63	57	60	63	57	59	±2·4
6	52	50	55	48	53	53	53	50	52	50	52	±2·1
Mean S.D. = ±3·2												
(b) *Duplicate analyses of the percentage duration of activity*												
1	49	47	52	47	47	48	47	52	45	48	48	±2·2
2	46	47	45	49	44	47	45	51	40	48	46	±3·0
3	17	19	19	16	18	16	19	19	18	18	18	±1·2
4	22	18	19	19	14	19	19	20	16	18	18	±2·1
5	60	57	61	59	60	59	58	60	62	59	60	±1·4
6	51	52	53	52	51	53	51	53	51	51	52	±0·9
Mean S.D. = ±1·8												

of these standard deviations is ± 3·2%. This was felt to be too great an error and therefore the standard deviations of duplicate analyses were calculated (Table IIb) and the mean of these standard deviations was considerably less (σ = ± 1·8). Thus with duplicate analyses it is unlikely that an error of more than 5% will occur (P = 1 : 100) and all figures for analyses in this study refer to the mean of two duplicate analyses.

RESULTS

COMPARISON OF MOTILITY OF PATIENTS WITH DUODENAL ULCER AND OF NORMALS Table III compares the analyses of the records from patients who had a duodenal ulcer and those of the normal subjects. The groups are compared at three levels; in the sigmoid colon, at the rectosigmoid area, and in the upper rectum. There are no significant differences between the groups.

COMPARISON OF MOTILITY AT DIFFERENT SEGMENTS OF PELVIC COLON Considering all the records from all the subjects, Table IV presents the analyses from the sigmoid, the rectosigmoid region, and the upper rectum. The mean duration of activity of the upper rectum does not differ significantly from that of the rectosigmoid or sigmoid but the amplitude of the

TABLE III

COMPARISON OF COLONIC MOTILITY OF PATIENTS WITH DUODENAL ULCER AND CONTROLS

	In Sigmoid Colon (20-25 cm.)		*At Rectosigmoid Area (15-20 cm.)*		*In Upper Rectum (10-15 cm.)*	
	Duodenal Ulcer	*Control*	*Duodenal Ulcer*	*Control*	*Duodenal Ulcer*	*Control*
Number	*14*	*18*	*10*	*16*	*8*	*15*
Duration of activity (%)	47·8 ± 21·5	54·3 ± 24·0	46·3 ± 23·9	52·5 ± 22·3	52·6 ± 26·6	64·6 ± 27·2
Mean amplitude of principal waves (cm. H_2O)	12·3 ± 7·3	10·7 ± 6·2	11·2 ± 9·2	12·0 ± 7·0	7·3 ± 4·5	7·0 ± 4·5
Total activity (1 × 2)	640 ± 393	640 ± 389	513 ± 403	689 ± 520	456 ± 432	501 ± 415
Waves of 20 cm. water pressure (%)	21·0 ± 21·0	20·8 ± 17·5	19·6 ± 21·8	19·5 ± 16·9	13·4 ± 16·1	9·2 ± 12·4
Time occupied by fast waves (%)	0·8 ± 1·7	2·4 ± 5·3	2·8 ± 4·0	5·3 ± 7·4	3·5 ± 4·5	5·9 ± 14·1

TABLE IV

COMPARISON OF MOTILITY OF SIGMOID, RECTOSIGMOID, AND UPPER RECTUM IN ALL SUBJECTS

	Sigmoid	*Rectosigmoid*	*Upper Rectum*
Number	*32*	*26*	*23*
Duration of activity (%)	51·4 ± 22·3	50·2 ± 22·5	60·7 ± 26·5
Mean amplitude of principal waves (cm. H_2O)	11·5 ± 6·5	11·7 ± 7·7	7·1 ± 4·5
Total activity (= 1 × 2)	640 ± 378	619 ± 467	485 ± 410
Waves of 20 cm. water pressure (%)	20·9 ± 18·5	19·5 ± 18·3	10·7 ± 13·6
Time occupied by fast waves (%)	1·7 ± 4·1	4·4 ± 6·2	5·0 ± 11·5
Frequency (waves/min.)	2·2	—	2·6

contractions in the rectum is significantly reduced. The frequency of the waves increases in the more caudal segments and the proportion of time occupied by waves of five per minute or more is also greater. These differences support the view that there is a functional distinction between the sigmoid and upper rectum.

DAY-TO-DAY VARIATION In one patient in whom studies were done on five occasions under the same conditions, the records obtained varied widely. The duration of activity varied between 10 and 90%. There was, however, close similarity in the features of the records during the active phases on each occasion. There was no obvious alteration in this patient's emotional reactions on the different occasions of study.

On the other hand, a second patient was studied on four occasions and produced very similar recordings on each occasion. The surprising feature in this patient was that the emotional state varied widely on different occasions of recording. On two occasions she was deeply disturbed emotionally and on one of these wept freely due to the discussion of distressing life experiences, while on the other two occasions she was resting quietly, without any evidence of emotional disturbance and slept for a time. The analysis of the two records obtained while the patient was under emotional stress is compared with the two records obtained while she was calm (Table V).

TABLE V

EFFECT OF MOOD ON COLONIC MOTILITY OF ONE SUBJECT

	Emotionally Disturbed (Mean of Two Experiments)	*Emotionally Calm (Mean of Two Experiments)*
Duration of activity (%)	85	94
Mean amplitude of principal waves (cm. H_2O)	24·4	20·2
Total activity	2,095	1,899
Waves of 20 cm. water pressure (%)	51	43
Time occupied by fast waves	2	4

Records were also analysed qualitatively.

PRINCIPAL WAVES The same features are seen in records from patients with duodenal ulcers and from normal subjects. A typical record is illustrated in Fig. 5. The principal wave represents a slow pressure change waxing and waning over approximately half a minute. The amplitude of these waves varies from less than 5 to more than 100 cm. of water pressure. Their distribution is irregular. Frequently, they occur in phases of activity which alternate with phases of relative inactivity. The duration and proportion of the active and inactive periods vary widely from subject to subject and in the same subject from time to time. Usually, however, the periodicity of these cycles is less than half an hour, although periods of total quiescence of up to one and a half hours, have been observed. Where the waves occur in

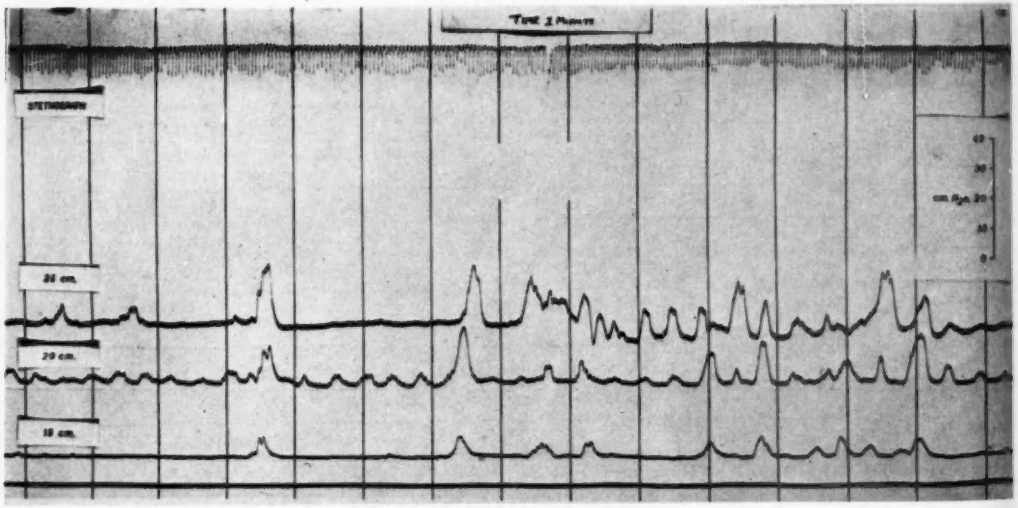

FIG. 5. *Normal colonic activity: principal waves. Top tracing = stethograph. Verticals = 1 minute intervals. Calibration in 10 cm. water steps.*

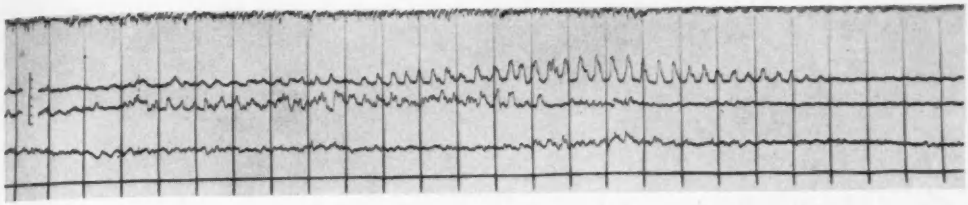

FIG. 6. *Normal colonic activity: gradual increase in amplitude of principal waves during phasic activity. Top trace = stethograph. Verticals = 1 minute intervals. Calibration in 10 cm. water steps.*

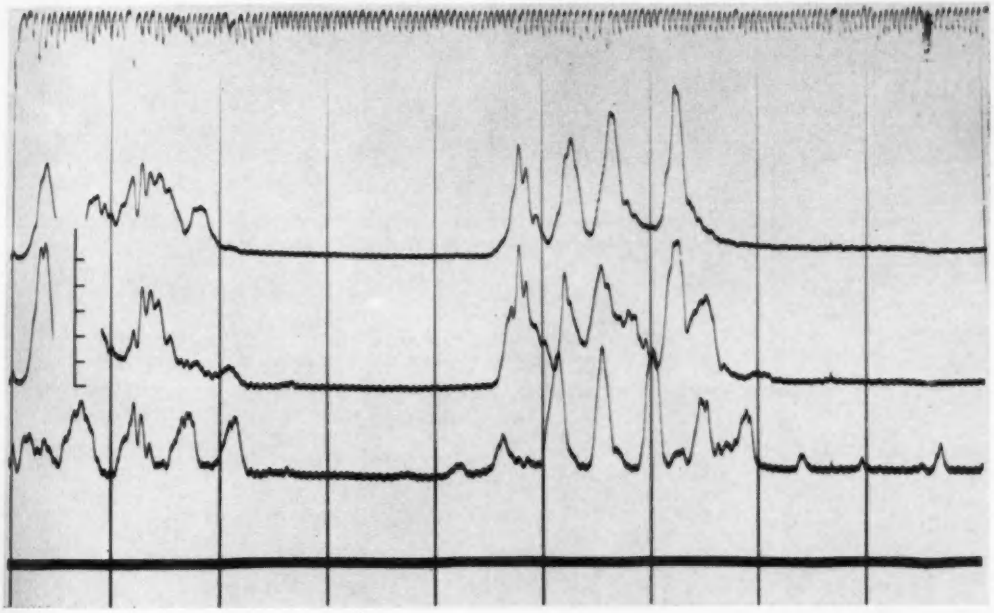

FIG. 7. *Normal colonic activity: short periods of high amplitude principal waves. Top tracing = stethograph. Vertical = 1 minute intervals. Calibration in 10 cm. water steps.*

FIG. 8. *Normal colonic activity: solitary principal waves. Top tracing = stethograph. Verticals = 1 minute intervals. Calibration in 10 cm. water steps.*

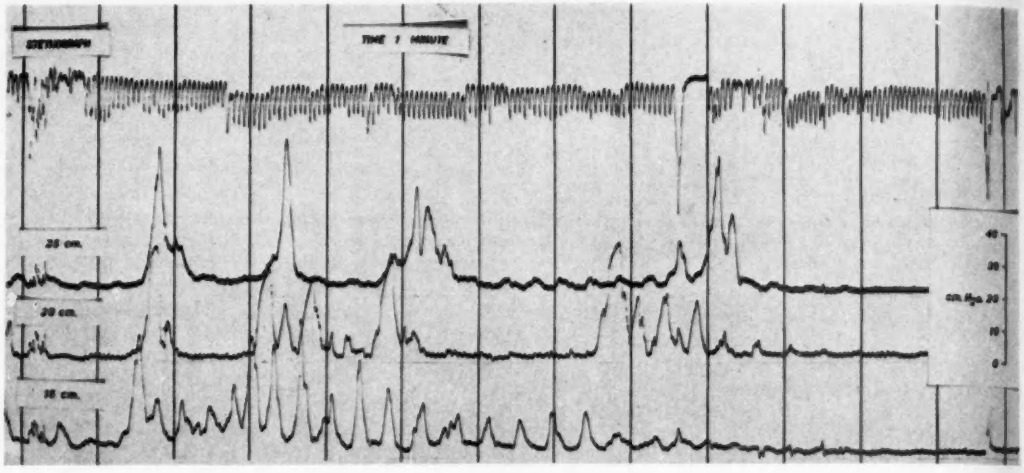

FIG. 9. *Normal colonic activity: independent segmental activity in simultaneous recording from points separated by 5 cm. Top tracing = stethograph. Verticals = 1 minute intervals. Calibration in 10 cm. water steps.*

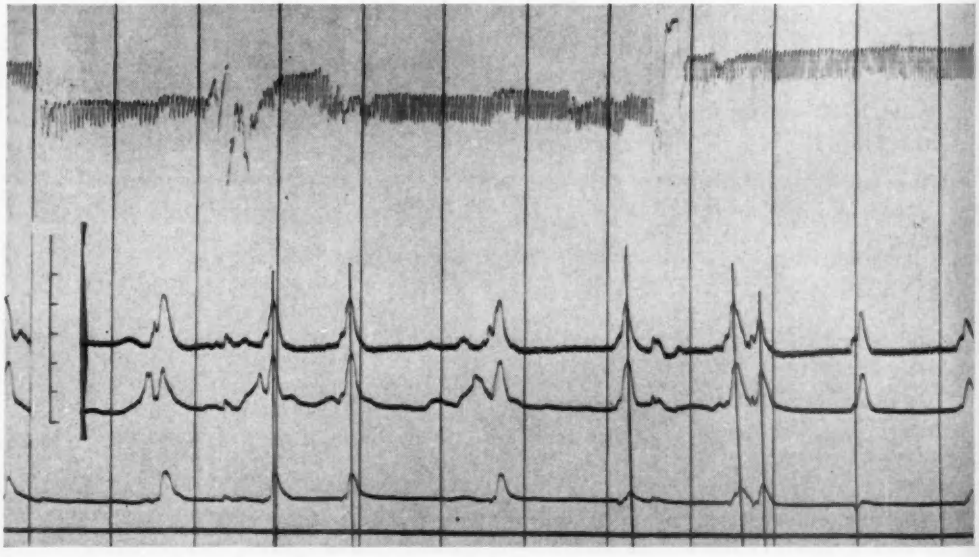

FIG. 10. *Normal colonic activity: progressive waves (lines have been drawn through waves which represent forward peristalsis). Top tracing = stethograph. Verticals = 1 minute intervals. Calibration in 10 cm. water steps.*

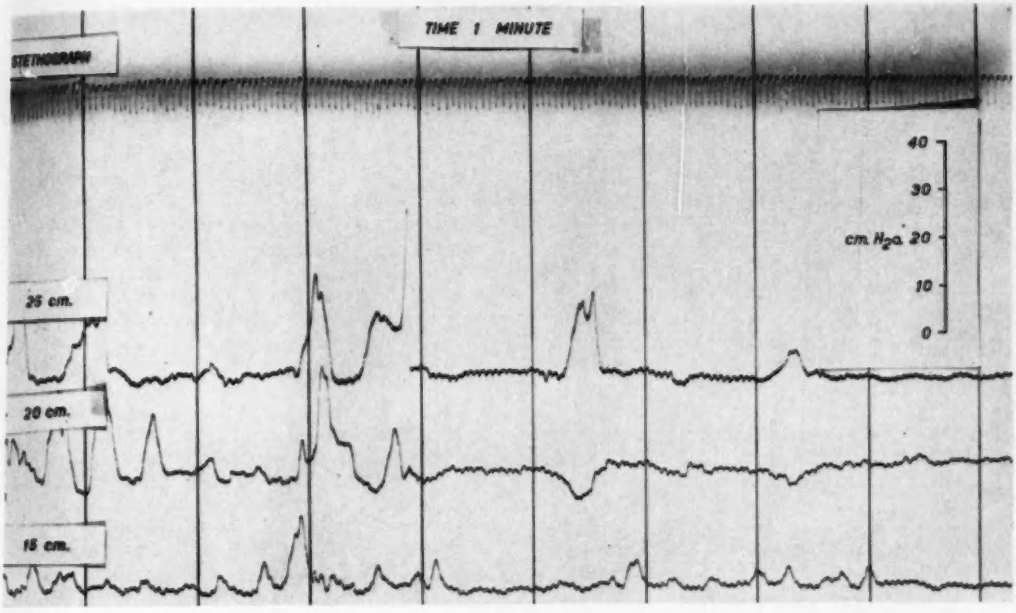

FIG. 11. *Normal colonic activity: relaxation (middle colonic tracing) associated with proximal contraction (upper colonic tracing). Top tracing = stethograph. Verticals = 1 minute intervals. Calibration in 10 cm. water steps.*

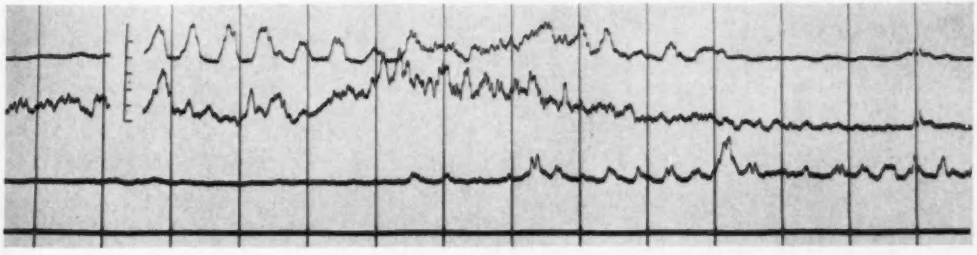

FIG. 12. *Normal colonic activity: rise in base line with superimposed colonic waves. Verticals = 1 minute intervals. Calibration in 10 cm. water steps.*

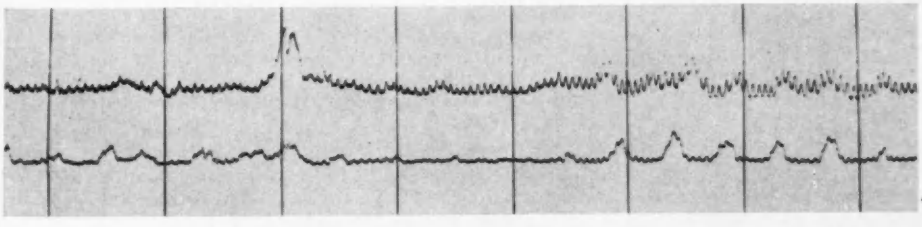

FIG. 13. *Normal colonic activity: alteration in amplitude of respiratory swing (upper tracing). Verticals = 1 minute intervals. Calibration in 10 cm. water steps.*

rhythmic sequence there is often a gradual build-up of amplitude followed by a gradual decline (Fig. 6). At other times, however, a short burst of waves of high amplitude may occur without any previous build-up (Fig. 7). Again independent solitary waves are not at all infrequent (Fig. 8, top trace). While the amplitude of these waves varies widely their frequency is remarkably constant at any given position in the pelvic colon, and when they occur in rhythmic sequence they seldom vary beyond the range of 1·8 to 2·7 per minute. They appear to correspond in form to the type described as type II (Templeton and Lawson, 1931).

SEGMENTAL ACTIVITY Simultaneous records from points separated by only short distances are frequently dissimilar, indicating independent segmental activity (Fig. 9). Whether or not the principal waves result in propulsion of the bowel contents will depend largely on the relationship of the pressures in neighbouring segments. Waves which are conducted over three segments are only rarely seen. One period in which such peristaltic waves were seen is illustrated in Fig. 10. This, however, was the only period in which such integrated progressive activity was seen in a record lasting five hours. The rate of progression in this case was 2·5 cm. per second. Waves representing reverse peristalsis at approximately the same rate also occur infrequently. Rarely distal relaxation associated with proximal contraction can be seen (Fig. 11).

FAST WAVES In the rectum there is an appreciable proportion of waves whose frequency varies from 5 to 10 cycles per minute. The amplitude of these fast waves is nearly always less than 20 cm. water pressure. They are very much less frequently seen in the sigmoid. They correspond to the type I described by Templeton and Lawson (1931).

CHANGES IN BASE LINE In some records there is a slow rise and fall of base line, the total duration of which may be 30 minutes or more, and the maximal amplitude is usually less than 5 cm. of water pressure. Base line changes of shorter duration also occur and frequently represent greater pressures, and are always associated with superimposed waves (Fig. 12). These changes in base line probably have some relationship to bowel 'tone'. The amplitude of the respiratory swings may also be a crude measure of tone because it varies considerably (Fig. 13, top trace). It presumably depends on the degree to which intra-abdominal pressure changes are transmitted into the lumen of the gut which in turn may be related inversely to the rigidity or tone of the bowel wall.

DISCUSSION

The pressures recorded in this study are the intraluminal pressures developed in the colon under resting physiological conditions. It is generally agreed that open tubes provide a true measure of intraluminal pressures (Quigley and Brody, 1950), and we have shown that open-ended tubes and miniature balloons give essentially similar results over the range of pressures generated in the colon. Indeed, in our experience, balloons are to be preferred to open-ended tubes for recording colonic motility if a 'tube' system is being employed. Even for the study of narrow segments such as the cardiac and pyloric sphincters balloons have been found to have certain advantages by Atkinson *et al.* (1957) and by Code and Schlegel (1958).

The analysis of motility records remains difficult and unstandardized. As Davidson *et al.* (1956) pointed out, the analysis into waves of Types I, II, III, and IV is an over-simplification, since all possible variations in duration and amplitude of waves occur. For example, Type III waves are defined as a rise in base line on which waves of Type I or II or both are superimposed (Adler *et al.*, 1941). However, the base line will rise when the rhythmic activity is sufficiently rapid or so powerful that the pressure generated by one contraction is not completely dissipated before the succeeding one begins. That is, this complex represents tetanic summation of a series of contractions. It seems therefore unrealistic to designate this feature as a distinct wave form. In addition, in some of the early papers, where sluggish water manometers were used, some of the forms described as Type III waves may have been the result of the sluggish response of the instrument resulting in a failure of the writing point to return to the base line before the start of another contraction.

On account of the very slow rate of contraction of the colon it appears to be a relatively inactive organ on radiological screening. This study, however, reveals activity of the colon for slightly more than half the time of recording under resting conditions. In this, our results agree with those of previous workers. Adler *et al* (1941) found activity for more than 50% of the time in the descending colon and Spriggs *et al.* (1951) found activity for 36% of the time in the pelvic colon of fasting subjects. It is interesting that there is agreement with these workers as they used large stimulating balloons to record colonic motility. Our results also agree with other workers using open-ended tubes. Davidson *et al.* (1956) found activity for 44% of the time in the sigmoid colon of children and Fink and Friedman (1960) showed that the range of activity was from 15 to 60% in the ascending colon in man.

In our records the greatest proportion of the time of activity is occupied by principal waves which do not appear to be progressive but to occur in an independent segmental manner. A contraction causing a high pressure in one segment associated with a lower pressure in a neighbouring segment will tend to propel the content of the bowel in the direction of this pressure gradient. If, however, the gradient reverses, the tendency will be for the contents to move in the opposite direction. Hence, the effect of these principal waves is to move the bowel content to and fro over a short length of bowel without any definite progression analwards. Barclay (1935) has shown that the rhythmic haustral contractions of the colon similarly move the contents from side to side and backwards and forwards over short distances, and Code, Hightower, and Morlock (1952) believe that the haustral contractions are the radiological analogue of the principal waves.

It is a well-known fact that sigmoidoscopically the greater bulk of faeces is usually found not in the rectum but at the rectosigmoid junction and above. Some mechanism must exist which prevents the forward progression of faeces. It has been shown that a high pressure zone does not exist at the rectosigmoid (Hill, Kelley, Schlegel, and Code, 1960). The finding that waves at a higher frequency were present for a longer proportion of the time in the upper rectum than in the sigmoid colon may be important because they might have the effect of protecting the rectum from overfilling. Spriggs *et al.* (1951) also found that 17% of the time of activity in the lower rectum was occupied by Type 1 or fast activity as opposed to 1% in the sigmoid. I have examined only a small number of records from the lower rectum but these few observations confirm the prominence of fast waves in this area. This increasing frequency of waves from sigmoid to anus may be a physiological mechanism whereby constant forward propulsion of faeces on to the sensitive areas for the final defaecation reflex in the lower rectum (Goligher and Hughes, 1951) is prevented.

The day-to-day variation of colonic motility in the same subject may be very considerable although the variation over several hours in the same day is much less marked. For this reason comparative studies of colonic activity in records obtained on different days must be interpreted with caution. The role of emotion in producing these day-to-day variations is problematical.

The pattern of colonic motility that emerges from this study confirms the experience of previous authors using large balloons and indicates an organ which is relatively active with approximately equal periods of activity and inactivity, the usual length of a complete cycle being about 30 minutes. In this it differs from the stomach where long periods of inactivity are broken only by short periods of rhythmic contraction, and from the small intestine in that the rate of contraction is very much slower.

The failure to demonstrate any difference in bowel motility between normals and patients with duodenal ulceration is at first sight surprising, but it should be emphasized again that our patients had no symptoms at the time of study. There is little evidence that the motility of the gastrointestinal tract is altered by the presence of a duodenal ulcer. Claims have been made that there is hypermotility of the stomach (Anderson, 1943) but Foulk, Code, Morlock, and Bargen (1954) and Smith, Texter, Stickley, and Barborka (1957) found no significant difference in small bowel motility between normals and patients with asymptomatic duodenal ulceration. The latter authors, however, did find significant changes in patients with active ulceration when pain was present. This present study shows no difference in the colonic motility in patients with asymptomatic duodenal ulceration but the presence of pain might affect colonic motility. Further work is required to establish this.

SUMMARY

The unstimulated motility of the sigmoid colon has been studied under physiological conditions using miniature balloons, which are advocated as the method of choice in studying this area of the bowel.

Normal patterns of colonic motility have been established and an analysis of the salient features of the motility records is presented.

On average the colon of normal subjects is active for 50% of the time of recording, but there is a wide range of activity in different persons and even in the same person from day to day.

The principal wave of the colon represents a slow independent segmental contraction lasting approximately one half minute, which does not have primarily a propulsive function.

There are differences in the frequency of contractions and the amplitude of the waves between the sigmoid colon and the rectum. The possible physiological significance is discussed.

Waves representing either forward peristalsis or reverse peristalsis are only occasionally seen.

There is no difference in the colonic motility of normal subjects and patients with asymptomatic duodenal ulceration.

I want to thank Dr. F. Avery Jones, Dr. T. D. Kellock, and the surgeons of St. Mark's Hospital for permission to study their patients.

The work was done while supported by a grant from the Medical Research Council held jointly by Dr. E. N. Rowlands and Mr. I. P. Todd, to whom I am indebted for helpful encouragement and advice at all stages in this work.

REFERENCES

Adler, H. F., Atkinson, A. J., and Ivy, A. C. (1941). A study of the motility of the human colon: An explanation of dysynergia of the colon, or of the "unstable colon". *Amer. J. dig. Dis.*, **8**, 197-202.

Anderson, W. F. (1943). Motility of the fasting stomach in health and disease. *Lancet*, **1**, 40-42.

Atkinson, M., Edwards, D. A. W., Honour, A. J., and Rowlands, E. N. (1957). Comparison of cardiac and pyloric sphincters: A manometric study. *Ibid*, **2**, 918-922.

Barclay, A. E. (1935). Direct x-ray cinematography, with a preliminary note on the nature of the non-propulsive movements of the large intestine. *Brit. J. Radiol.*, **8**, 652-658.

Bockus, H. L. (1944). *Gastro-enterology*, Vol. II, p. 497. Saunders, Philadelphia.

Code, C. F., Hightower, N. C., Jr., and Morlock, C. G. (1952). Motility of the alimentary canal in man. *Amer. J. Med.*, **13**, 328-351.

——, and Schlegel, J. F. (1958). The pressure profile of the gastro-esophageal sphincter in man: An improved method of detection. *Proc. Mayo Clin.*, **33**, 406-414.

Connell, A. M., and Rowlands, E. N. (1960). Wireless telemetering from the digestive tract. *Gut*, **1**, 266-272.

Davidson, M., Sleisenger, M. H., Almy, T. P., and Levine, S. Z. (1956). Studies of distal colonic motility in children: Non-propulsive patterns in normal children. *Pediatrics*, **17**, 807-819.

Edwards, D. A. W., and Rowlands, E. N. (1960). An air-filled metal capsule optical manometer system. In *Medical Electronics*, ed. C. N. Smyth, pp. 307-312. Iliffe, London. Proc. 2nd International Conference on Medical Electronics, Paris 1959.

Fink, S., and Friedman, G. (1960). The differential effect of drugs on the proximal and distal colon. *Amer. J. Med.*, **28**, 534-450.

Foulk, W. T., Code, C. F., Morlock, C. G., and Bargen, J. A. (1954). A study of the motility patterns and the basic rhythm in the duodenum and upper part of the jejunum of human beings. *Gastroenterology*, **26**, 601-611.

Goligher, J. C., and Hughes, E. S. R. (1951). Sensibility of the rectum and colon; its role in the mechanism of anal continence. *Lancet*, **1**, 543-548.

Hill, J. R., Kelley, M. L., Schlegel, J. F., and Code, C. F. (1960). Pressure profile of the rectum and anus of healthy persons. *Dis. Colon Rect.*, **3**, 203-209.

Jones, F. A., and Gummer, J. W. P. (1960). *Clinical Gastroenterology*, p. 333. Blackwell Scientific Publications, Oxford.

Quigley, J. P., and Brody, D. A. (1950). "Digestive Tract: Intralumen pressures: Gastrointestinal propulsion, gastric evacuation, pressure-wall tension relationships." In Glasser, O., *Medical Physics*, Vol. 2., p. 280. The Year Book Publishers, Chicago.

Rowlands, E. N., Honour, A. J., Edwards, D. A. W., and Corbett, B. D. (1953). Metal-capsule optical manometer for measuring pressure in the human gut with an open-ended tube. *Clin. Sci.*, **12**, 299-306.

Smith, H. W., Texter, E. C., Jr., Stickley, J. H., and Barborka, C. J. (1957). Intraluminal pressures from the upper gastrointestinal tract. II. Correlations with gastroduodenal motor activity in normal subjects and patients with ulcer distress. *Gastro-enterology*, **32**, 1025-1049.

Spriggs, E. A., Code, C. F., Bargen, J. A., Curtiss, R. K., and Hightower, N. C. (1951). Motility of the pelvic colon and rectum of normal persons and patients with ulcerative colitis. *Ibid*, **19**, 480-491.

Templeton, R. D., and Lawson, H. (1931). Studies in the motor activity of the large intestine: Normal motility in the dog, recorded by the tandem balloon method. *Amer. J. Physiol.*, **96**, 667-676.

Potassium replacement therapy

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SYNOPSIS Potassium replacement is often an important therapeutic measure, and the advantages of effervescing potassium-containing granules are put forward in this article.

Potassium depletion is a very real additional danger to patients already ill from other causes. Potassium ion may be rapidly lost from the alimentary tract, as in vomiting and diarrhoea, or from the kidney when diuretics are used in diseases associated with hyperaldosteronism such as chronic liver disease or congestive cardiac failure.

The treatment of potassium depletion holds particular difficulties. When given in tablet form absorption is capricious because of the varied times taken for the tablets to disintegrate, and when intestinal transit is rapid the tablets not infrequently appear unaltered in the faeces or ileostomy bag. The bitter taste of potassium-containing mixtures makes them unpalatable to many patients and may lead to anorexia, nausea, and vomiting. These side-effects are particularly incommoding in conditions where the clinician aims to stimulate or at least maintain the appetite to combat malnutrition. The following prescription taken in an equal quantity of Ribena syrup is more palatable than most:

Pot. chloride.....1 g.
Syrup of raspberry.....2 ml.
Chloroform water.....to 15 ml.

Nevertheless its use is not without the risk of gastro-intestinal disturbance.

At the Royal Free Hospital an effervescent potassium tablet has been developed (Hadgraft, 1960) which is both palatable and effective. This is composed of potassium bicarbonate, potassium acid tartrate, and anhydrous citric acid. It contains 6·5 mEq. of potassium per tablet. One practical drawback has been the slowness of these tablets to go into solution and their liability to leave behind undissolved residues in the glass. Examination of the glass revealed up to 0·3 mEq.K remaining after tablets had been given to patients routinely by the nursing staff.

We prefer to use effervescent potassium-containing granules for this reason and also because their superior effervescence makes the granules rather more palatable. The formula used is:

Potassium bicarbonate.....50 g.
Potassium acid tartrate.....30 g.
Citric acid.....10 g.
Sodium saccharin.....0·5 g.
Sucrose powder.....20 g.

The granules, made by the method described in the British Pharmaceutical Codex, 1934, are dispensed either

in 4 g. quantities in sealed packages to be opened at the bedside or in airtight screw cap containers, from which they may be dispensed by the teaspoonful (3-4 g.).

Four grammes of these granules containing 26 mEq. of potassium dissolve rapidly in a glass of water making a pleasant effervescent drink which may be flavoured with fruit juice if so desired. Solution is complete within 30 seconds and the potassium residues remaining in the glass amount to less than 0·1 mEq.

A dose of 8 to 12 g. of these granules daily causes a minimum of gastric disturbance yet provides an intake of potassium large enough to combat potassium depletion in all except the most extreme clinical contingencies. Reliability of solution and absorption commends the use of this preparation in preference to enteric-coated potassium tablets.

REFERENCE

Hadgraft, J. W. (1960). *Pharm. J.*, **184**, 277-279.

A method of measuring gastric pressure during vagotomy

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During the use of the test for completeness of gastric vagotomy described by Burge and Vane (1958) some difficulty has been found with the change in gastric pressure caused by respiration.

The test consists of stimulating the vagus nerves with an electrode placed around the oesophagus. This stimulation produces a rise in gastric pressure which varies with the size of the nerves present. Using an ordinary water manometer and the stomach being distended with air to a pressure of 20 cm.H_2O, respirations cause a rise in gastric pressure of between 1 and 3 cm. The heart beat may also cause a slight rise in pressure. On stimulation, the average vagal nerve trunk produces a rise of between 4 and 10 cm. A small nerve, however, may produce an increase of only 1 cm. and is not easy to observe if respiration is present.

It was decided to attempt to make use of the fact that the respiratory rise only lasts a few seconds whereas that due to the presence of an intact nerve lasts some 20 seconds. An apparatus has been devised in which the quick rise and fall in gastric pressure caused by respiration is not recorded and yet remains sensitive to the slightest change in gastric tone caused by vagal stimulation.

THE APPARATUS

In its simplest form the apparatus consists of two reservoirs of water, (A) and (B), connected together with a plastic tube. The side tubes over which the plastic tube fits have very narrow bores to produce a high fluid resistance. A glass tube (C), of about 5 mm. bore, dips into reservoir (A). A glass capillary tube (D) dips into reservoir (B). This tube is drawn out to a fine bore at its lower end. Both tubes are fitted with scales, that on tube (C) being marked to indicate a pressure of 20 cm.H_2O. The scale on tube (D) is graduated in centimetres and may be slid up and down the tube.

In use the stomach is distended with air to a pressure of 20 cm. of water as shown on tube (C). The scale on tube (D) is then adjusted until the zero corresponds with the level of fluid in the tube. The vagi are then stimulated. Tube (C) will show at once all pressure changes due to respiration, heart beat, and gastric tone. Only the sustained

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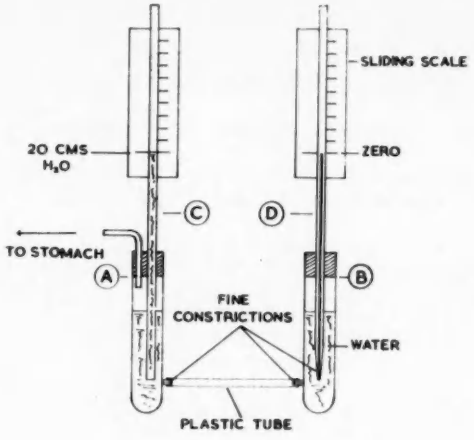

rise in pressure caused by vagal stimulation will produce a rise in tube (D). Any increase in pressure in this tube may be regarded as demonstrating the presence of a vagal nerve.

It would seem possible that this simple device might find other applications where it is desired to measure changes in pressure without interference from extraneous movements.

The apparatus is easily made in any hospital laboratory from two 6 in. × 1 in. test-tubes and various pieces of glass and plastic tubing.

My thanks are due to Mr. Harold Burge for his encouragement and to Dr. L. Chapman for his help in making the prototype.

REFERENCE

Burge, H., and Vane, J. R. (1958). *Brit. med. J.*, **1**, 615.

The March 1961 Issue

THE MARCH 1961 ISSUE CONTAINS THE FOLLOWING PAPERS

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Primary Crohn's disease of the colon and rectum[1]

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SYNOPSIS Crohn's disease of the large bowel has been increasingly recognized and this paper presents a clinical and pathological study of 45 patients in whom the disorder was confined entirely to the large intestine. The clinical and pathological factors are contrasted with those of 86 patients with regional ileitis and of 200 patients with ulcerative colitis. Compared with regional ileitis the disease occurred in an older age group, had a different sex distribution, and a higher incidence of perianal and rectovaginal fistulae. A high incidence of recurrent disease following surgical treatment, found in the patients with regional ileitis, was not found in the patients with primary Crohn's disease of the large intestine. The incidence of the disease relative to ulcerative colitis was approximately 6%. The disease had the same sex distribution as ulcerative colitis but occurred in an older age group. The disease was usually of gradual onset and continuous course, and periods of freedom lasting a month or more were uncommon. The patients came to surgery much earlier than patients with ulcerative colitis.

This study began with the examination of a small number of specimens of the large intestine showing many of the pathological features of regional ileitis described by Hadfield (1939), Warren and Sommers (1948), and Rappaport, Burgoyne, and Smetana (1951). The terminal ileum, however, was not involved in any of these cases and the usual preoperative diagnosis was idiopathic ulcerative colitis.

Although the first 13 cases of regional ileitis reported by Crohn, Ginzburg, and Oppenheimer in 1932 affected only the terminal ileum it was soon appreciated that the proximal ileum and jejunum could also be involved (Harris, Bell, and Brunn, 1933), and that the disease might be confined entirely to the jejunum (Brown, Bargen, and Weber, 1934). Involvement of the colon was first reported by Colp in 1934, and later by Donchess and Warren (1934), by Brown *et al.* (1934), by Crohn and Rosenak (1936), and by others.

Recognition of the disease arising primarily in the large intestine has been retarded by a confusion in nomenclature, by the difficulty in clinical distinction from ulcerative colitis, and by the failure to base published series on adequately studied pathological material. Some pathologists do not distinguish between regional ileitis, regional colitis, and ulcerative colitis, regarding them all as variants of an essentially similar process manifesting itself in different areas of the bowel (Lumb, 1951; Goldgraber, Kirsner, and Palmer, 1959; Lumb and Protheroe, 1958). However, the pathological features of regional ileitis and ulcerative colitis are sufficiently dissimilar for a clear-cut distinction to be made between these two conditions in the vast majority of cases (Rappaport *et al.*, 1951; Warren and Sommers, 1954; Lockhart-Mummery and Morson, 1960).

The confusion in nomenclature has arisen from the use of the words 'regional' or 'segmental' colitis to cover all cases of non-specific chronic inflammation of the large intestine in which the rectum, rectosigmoid, and terminal ileum are not primarily involved (Neuman, Bargen, and Judd, 1954). Defined in this way the term 'regional' colitis embraces at least three different types of disease, namely, ulcerative colitis, secondly a disease with pathological features similar to regional ileitis, and thirdly a disease which fails to show the characteristic pathological features of either regional ileitis or ulcerative colitis. This last type probably represents a group of unrecognized disorders.

To overcome the difficulty of nomenclature Harris *et al.* used the term 'cicatrizing enteritis' to cover all cases with similar clinical and pathological features to the cases of regional ileitis described by Crohn *et*

[1]Based on a lecture given in a series of talks on 'Current Pathology' at Westminster Medical School on 27 February 1961.

al. Crohn and his colleagues, however, use the term 'granulomatous colitis' to describe cases of regional ileitis with colonic involvement in which the pathological features of the disease in the colon and ileum are identical (Crohn and Yarnis, 1958). Although 'cicatrizing enteritis' and 'granulomatous colitis' are useful terms they do not adequately describe the important and characteristic features of this disease. Cicatrizing enteritis can be found in a number of chronic inflammatory disorders (Lumb, 1951), and granulation tissue may be found in the superficial submucosa in the healing phase of ulcerative colitis (Dukes, 1954). Wells (1952), describing cases of regional colitis characterized by fibrosis, non-caseating tubercles, and multinucleated giant cells, called the condition 'Crohn's disease of the colon'. Because this eponymous title conveys a much fuller meaning than any other term previously employed it has become justly popular with British surgeons and pathologists, and is now an accepted part of our terminology (Dukes and Lockhart-Mummery, 1957; Brooke, 1959; Watkinson, Thompson, and Goligher, 1960; Lockhart-Mummery and Morson, 1960; Nevin, 1961).

Although Crohn's disease of the colon associated with regional ileitis is now a well recognized clinical and pathological entity, very few cases of Crohn's disease arising primarily in the large intestine and in which the small intestine was apparently normal have been reported in the literature. In retrospect some of the cases reported by Moynihan (1907), Robson (1908), Braun (1909), Dalziel (1913), Läwen (1914), Tietze (1920), and Moschcowitz and Wilensky (1923) were probably examples of primary Crohn's disease of the large intestine. Well-documented case reports of this type of disease have been published by James (1938) and by Castleman (1944). Brooke (1959) reported seven cases, in two of which the disease recurred in the small intestine after total proctocolectomy. Lockhart-Mummery and Morson (1960) have recently described 18 cases of primary Crohn's disease of the large intestine and seven cases of regional ileitis with large intestinal involvement.

It is sometimes assumed that primary Crohn's disease of the large intestine occurs in the same type of patient as regional ileitis, and that the clinical behaviour of the disease in the large intestine is similar to that in the small. Goligher (1961) states that there is no reason to suppose Crohn's disease loses its notorious predilection to recurrence because it happens to have transferred its habitat from the small intestine to the large. Dukes (1959), however, believes that a careful distinction should be made between cases of primary Crohn's disease of the large intestine and cases of regional ileitis with or without colonic involvement. This study was undertaken to see if this distinction was worth making, to assess the incidence of primary Crohn's disease of the large intestine relative to ulcerative colitis, and to compare the clinical and pathological features of these cases with cases of regional ileitis and ulcerative colitis.

CASES STUDIED

This study is based on 45 patients suffering from primary Crohn's disease of the large intestine treated by surgical resection from whom parts or all of the large intestine were available for pathological examination. Only patients in whom the small intestine was shown to be free from disease by radiological examination, inspection at operation, and histological examination of the resected portion of the terminal ileum when it was present in the operation specimen, were included in this group. There was no evidence of active tuberculosis or sarcoidosis, and no acid-fast bacilli could be found in the stools or in sections from lesions in the large intestine and regional lymph nodes. Strict histological criteria were used in the diagnosis of Crohn's disease, and only those patients with a non-specific chronic inflammatory reaction affecting the submucosa, muscle coats and subserosa, associated with fibrosis and the presence of non-caseating nodules of epithelioid histiocytes in the wall of the large intestine or regional lymph nodes, were included.

For comparison a study was made of 86 patients with regional ileitis who had been treated by surgical resection and from whom operation specimens were available for pathological examination.

To assess the incidence of primary Crohn's disease of the large intestine relative to ulcerative colitis a study was made of 200 consecutive patients with proved ulcerative colitis treated by colectomy at the Gordon Hospital, London, and the number of patients with primary Crohn's disease of the large intestine treated by surgical resection at the same hospital over the same period of time was determined. Only those patients with the characteristic pathological features of ulcerative colitis described by Dukes (1954) and by Warren and Sommers (1954) were included in this group.

The clinical records of all the patients included in this study were analyzed, and follow-up studies were made wherever possible.

RESULTS

AGE AND SEX INCIDENCE The age and sex incidence of the patients in this study are shown in Table I. (The age given is the age at onset of symptoms.) Whilst regional ileitis occurred more commonly in

TABLE I

AGE AND SEX DISTRIBUTION

Age (years)	*Regional Ileitis*			*Ulcerative Colitis*			*Primary Crohn's Disease of Large Intestine*		
	Males	*Females*	*Total*	*Males*	*Females*	*Total*	*Males*	*Females*	*Total*
0-10	2	2	4	4	3	7	0	1	1
11-20	19	10	29	8	18	26	4	5	9
21-30	17	14	31	29	42	71	3	2	5
31-40	9	2	11	20	23	43	5	0	5
41-50	5	5	10	17	20	37	1	4	5
51-60	1	0	1	2	8	10	3	2	5
61-70	0	0	0	4	2	6	2	9	11
71-80	0	0	0	0	0	0	0	4	4
Total	53	33	86	84	116	200	18	27	45

males than in females, ulcerative colitis and primary Crohn's disease of the large intestine occurred more commonly in females than in males. Primary Crohn's disease of the large intestine was found in an older age group than either regional ileitis or ulcerative colitis.

FAMILIAL INCIDENCE Nine patients with ulcerative colitis gave a history of the disease occurring in other members of their families. One woman with regional ileitis had a brother suffering from the same disease. One man with primary Crohn's disease of the large intestine had a brother suffering from regional ileitis.

SYMPTOMS Although diarrhoea, melaena, abdominal pain, weight loss, and fatigue are symptoms common to ulcerative colitis, primary Crohn's disease of the large intestine, and regional ileitis with colonic involvement, a detailed analysis of the data obtained in this study draws attention to certain differences in their symptoms.

Patients with primary Crohn's disease of the large intestine were treated by surgical resection earlier than patients with ulcerative colitis. The number of patients with this disorder undergoing surgical resection within five years of the onset of the disease was 77·8% compared with 48% in ulcerative colitis, and after 10 years was 8·9% compared with 26·5% in ulcerative colitis.

An acute onset was found more commonly in patients with ulcerative colitis (29·5%) than in patients with primary Crohn's disease of the large intestine (11·1%). Periods of freedom from disease lasting a month or more, which were found in more than half the patients with regional ileitis and ulcerative colitis, were found in only six of the 45 patients with primary Crohn's disease of the large intestine.

All the patients with ulcerative colitis suffered from diarrhoea, although at the onset several of these patients complained of constipation. Diarrhoea occurring in patients with regional ileitis or primary Crohn's disease of the large intestine was usually less severe than that occurring in the patients with ulcerative colitis. Eighteen patients with regional ileitis and four patients with primary Crohn's disease of the large intestine had no diarrhoea.

Despite the frequent finding of occult blood in the stools five patients with ulcerative colitis (2·5%), eight patients with primary Crohn's disease of the large intestine (17·8%), and 49 patients with regional ileitis (56·9%) had not noticed any blood in their stools.

EXAMINATION AND INVESTIGATIONS A striking difference was found in the incidence of perianal and rectovaginal fistulae in the three types of disease. These fistulae were found in nearly two-thirds of the patients with primary Crohn's disease of the large intestine, and nearly one-third of the patients with regional ileitis. Compared with ulcerative colitis the characteristic features of the fistulae in regional ileitis and primary Crohn's disease of the large intestine were their indolent appearance, their multiplicity, and their frequency of recurrence after surgical treatment.

A pathological examination was made of the tissues removed from just over half the operations on these fistulae. Although multinucleated foreign body type giant cells were frequently seen in this material, non-caseating tubercles with Langhans type giant cells were found only in material from patients with regional ileitis or primary Crohn's disease of the large intestine.

At sigmoidoscopy the rectal mucosa appeared abnormal in 198 of the 200 patients with ulcerative colitis, in 31 of the 45 patients with primary Crohn's disease of the large intestine, and in 24 of the 86 patients with regional ileitis. Non-caseating tubercles with giant cells were found in the rectal biopsies from a fifth of the patients with rectal disease due to

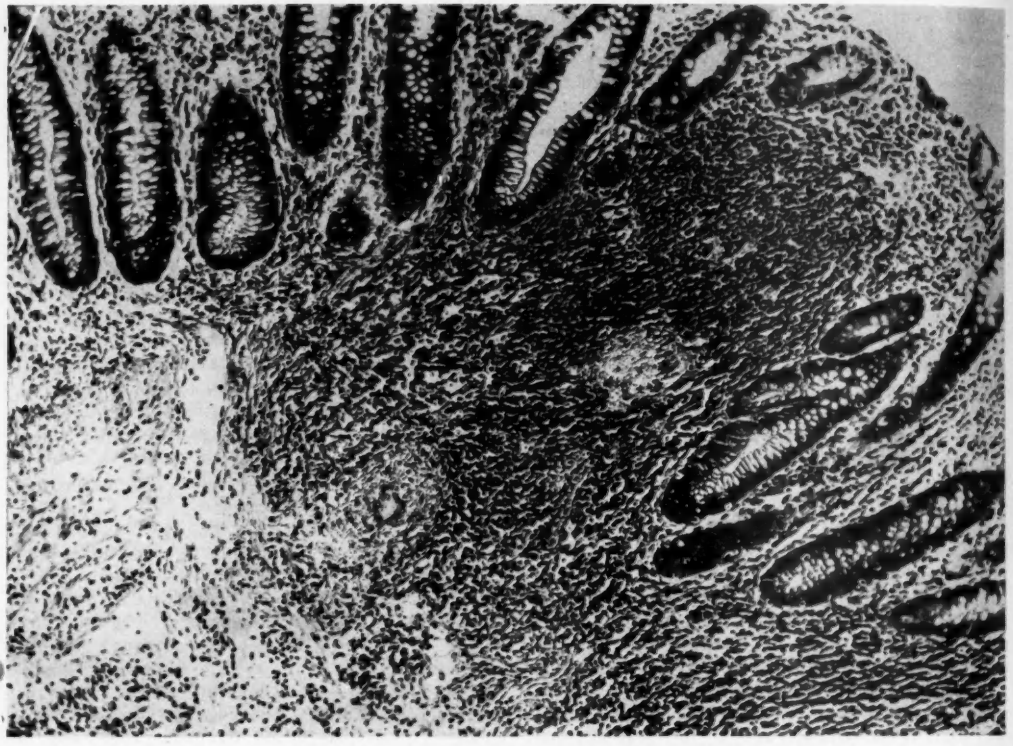

FIG. 1. *Crohn's disease. Rectal biopsy showing tubercles and giant cells. x 120.*

regional ileitis or primary Crohn's disease of the large intestine (Fig. 1).

Painful, swollen joints were found in seven of the 45 patients with primary Crohn's disease of the large intestine, in seven of the 86 patients with regional ileitis, and in 19 of the 200 patients with ulcerative colitis. Ankylosing spondylitis was found in two patients with regional ileitis and in two patients with ulcerative colitis. Erythema nodosum was seen in two patients with primary Crohn's disease of the large intestine, in five patients with regional ileitis, and in seven patients with ulcerative colitis.

Normal haemoglobin levels, above 11·5 g. per 100 ml., were found in 43·2% of the patients with primary Crohn's disease of the large intestine, in 43·2% of the patients with regional ileitis, but in only 26% of the patients with ulcerative colitis. Haemoglobin levels below 60% (Haldane) were found in 9·1% of the patients with primary Crohn's disease of the large intestine, in 9·5% of the patients with regional ileitis, and in 21·6% of the patients with ulcerative colitis. The erythrocyte sedimentation rate was found to be a useful indicator of the activity of all three diseases. We were unable to demonstrate any special association between the ABO and Rh anti-D blood groups and these three types of disease.

DISTRIBUTION OF LESIONS The distribution of the lesions in the small and large intestine in the patients with regional ileitis, ulcerative colitis, and primary Crohn's disease of the large intestine when surgical resections were first performed are shown in Table II.

Skip lesions were found in 43·3% of the patients with regional ileitis involving the large intestine (Fig. 2), in 20% of the patients with primary Crohn's disease of the large intestine (Fig. 3), and in only 1% of the patients with ulcerative colitis.

Involvement of the large intestine, found in 49 of the 86 patients with regional ileitis, was of two types. In the first type the lesions in the large intestine were in continuity with those in the terminal ileum and they appeared to be spreading along the large

FIG. 2. *Regional ileitis with skip lesion in descending colon.*

FIG. 3. *Primary Crohn's disease of the large intestine with small intervening areas of normal mucosa.*

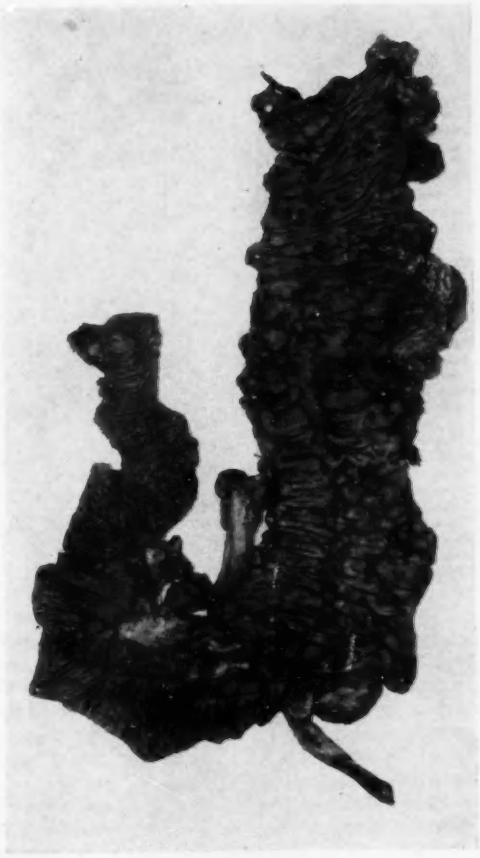

FIG. 4. *Regional ileitis with spread to caecum and ascending colon, viewed from behind.*

TABLE II

DISTRIBUTION OF LESIONS IN PRIMARY CROHN'S DISEASE OF LARGE INTESTINE, IN REGIONAL ILEITIS, AND IN ULCERATIVE COLITIS

Distribution	*No of Patients*		*No. with Skip Lesion*
Rectum to and including			
Sigmoid colon	5		0
Descending colon	4		1
Transverse colon	6		1
Ascending colon	1		0
Caecum	13	29	2
Caecum to and including			
Ascending colon	2		0
Transverse colon	1		1
Descending colon	3		1
Sigmoid colon	1	7	0
Ascending and transverse colon		1	1
Transverse to sigmoid colon		1	0
Caecum, sigmoid, and rectum		2	2
Transverse colon		3	0
Sigmoid colon		2	0
	Total	45	9
Ileum to and including			
Caecum	11		0
Ascending colon	5		0
Transverse colon	5		2
Descending colon	3		0
Sigmoid colon	3		3
Rectum	7	34	1
Ileum with skip to sigmoid and rectum		13	13
Ileum and caecum, with skip to sigmoid and rectum		2	2
	Total	49	21
Rectum to and including			
Sigmoid colon	4		0
Descending colon	10		0
Transverse colon	11		0
Ascending colon	17		0
Caecum	115		2
Terminal ileum	41	198	0
Caecum to descending colon		1	0
Transverse colon		1	0
	Total	200	2

intestine in a caudal direction (Fig. 4). In the second type lesions were found in the sigmoid colon and rectum which appeared to have been spread by a surface contact between the terminal ileum and the rectosigmoid. In several of these cases the surgeons found the terminal ileum stuck to the sigmoid colon by fibrous adhesions.

The incidence of segmental colitis varied with the different types of disease. It was uncommon in ulcerative colitis, but occurred in nearly one-third of the patients with primary Crohn's disease of the large intestine and in more than half the patients with regional ileitis involving the large intestine.

TREATMENT Ten patients with primary Crohn's disease of the large intestine were treated by total proctocolectomy, nine by subtotal proctocolectomy, nine by total colectomy, and 17 by subtotal colectomy. The disease was incompletely removed in 10 cases.

Twelve patients with regional ileitis were treated by resection of the diseased terminal ileum, 65 by removal of the terminal ileum with right hemicolectomy, and eight by removal of the terminal ileum with total colectomy. One patient was treated by the removal of a skip lesion in the sigmoid colon, the affected terminal ileum being left behind.

Most of the patients with ulcerative colitis were treated by total colectomy and ileo-rectal anastomosis, as described by Aylett (1960). Subtotal colectomy was performed in 11 cases, and palliative abdominoperineal excision of the rectum for advanced carcinoma in three cases.

MACROSCOPIC APPEARANCES There were no differences in the macroscopic or microscopic appearances of the large intestinal lesions in patients with regional ileitis and those of primary Crohn's disease of the large intestine.

Certain gross features helped to distinguish specimens of primary Crohn's disease of the large intestine from specimens of ulcerative colitis. A segmental distribution and the presence of skip lesions occurred much more frequently in Crohn's disease than in ulcerative colitis. The widespread, ragged, superficial ulceration seen in acute ulcerative colitis (Fig. 5) was not seen in the specimens of Crohn's disease, in which the ulcers were usually smaller, more elongated, and had a somewhat fissured appearance. The ulcers of Crohn's disease extended in a serpiginous fashion along the lumen of the bowel (Fig. 6), and occasionally branched or crossed each other to give a reticular pattern (Fig. 7). The mucosa between these ulcers was oedematous, the combination of fissures with a swollen mucosa producing a typical 'cobblestone' appearance.

Mucosal tags projecting into the lumen of the large intestine were a characteristic feature of many of the specimens of ulcerative colitis (Fig. 8). They were not found in the specimens from patients with primary Crohn's disease of the large intestine.

Dilatation of the colon was found in 24 specimens from patients with acute ulcerative colitis. No dilatation was found in any of the specimens from patients with Crohn's disease of the large intestine.

Serosal inflammation was a constant finding in the specimens from patients with primary Crohn's disease of the large intestine, and tiny greyish-white nodules, the size of a pin head, were seen on the serosal surface of six of these specimens. These serosal nodules were not found on the specimens from patients with ulcerative colitis. During operations on patients with ulcerative colitis the surgeons frequently noticed congestion and apparent inflammation of the serosal surface of the colon, but by the time most specimens reached the laboratory these features were no longer obvious. Specimens from

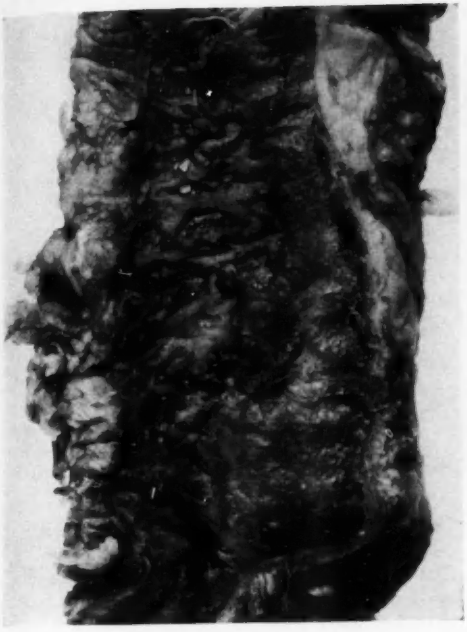

FIG. 5. *Ulcerative colitis showing widespread ragged superficial ulceration.*

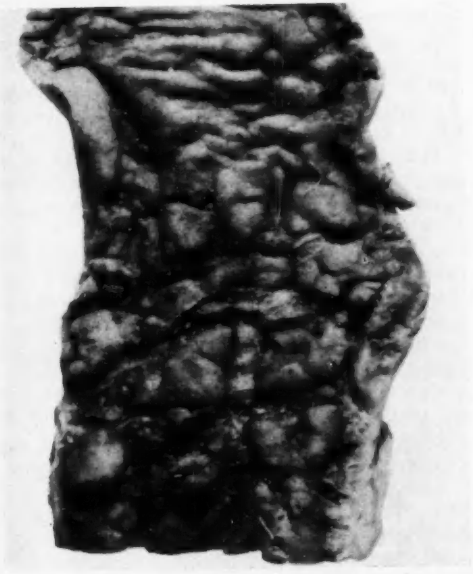

FIG. 6. *Primary Crohn's disease of the large intestine. Close-up view of ulcers in the descending colon.*

FIG. 7. *Primary Crohn's disease of the sigmoid colon and rectum showing the characteristic 'cobblestone' appearance of the intestinal mucosa.*

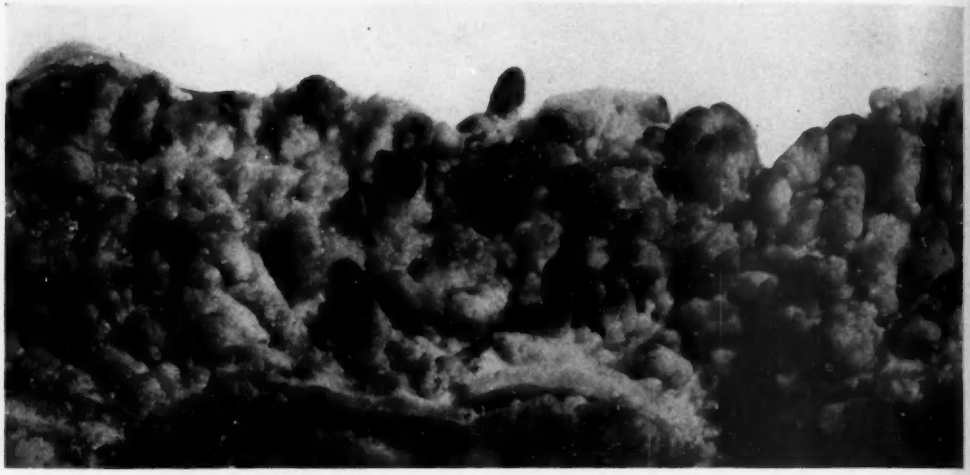

FIG. 8. *Ulcerative colitis with mucosal tags in the transverse colon.*

patients with acute ulcerative colitis, however, invariably showed an acute inflammatory reaction of the serosal surface, and perforations were occasionally seen.

Internal fistulae were found in two patients with primary Crohn's disease of the large intestine and in 16 patients with regional ileitis. No internal fistula was found in the patients with ulcerative colitis.

Enlarged lymph nodes were found in 51·1% of the specimens of primary Crohn's disease of the large intestine, 68·9% of the specimens of ulcerative colitis, and 82·7% of the specimens of regional ileitis. The largest nodes were found in specimens from patients with ulcerative colitis and they frequently misled the surgeons into suspecting the presence of an associated carcinoma. Carcinomas were not found in any of these cases, however, and the large lymph nodes simply contained widely dilated sinusoids packed with chronic inflammatory cells.

MICROSCOPIC APPEARANCES Sections from the specimens of primary Crohn's disease of the large intestine showed a chronic inflammatory reaction affecting the submucosa, muscle coats, and subserosa. Some damage to the muscle coats was invariably found, and fibrous tissue was seen throughout the submucosa, and often in the muscle coats and subserosa as well. The muscle coats were usually considerably thickened, and the myenteric plexus and ganglion cells were prominent. Focal collections of lymphocytes were seen in the submucosa, muscle coats, and subserosa. Fissures extending into and often penetrating the muscle coats were frequently found, and were the basis for the formation of internal fistulae.

Foreign body type giant cells were seen in areas of ulceration and around abscesses in the wall of the large intestine. Non-caseating nodules of epithelioid histiocytes containing small multinucleated giant cells were found typically only in areas free from mucosal ulceration. These nodules were seen in the submucosa, muscle coats, subserosa, and regional lymph nodes. They were rarely found in enlarged lymph nodes. Schaumann bodies were seen in nodules of epithelioid cells in the large intestine of one patient with primary Crohn's disease of the large intestine and in the pericolic lymph nodes of two other patients with this disorder.

Brunner-type glands were found in specimens of terminal ileum from 29 patients with regional ileitis (Fig. 9). They were found only in areas of mucosal regeneration, and were often only partly stained by the indulin-mucicarmine technique of Kawel and Tesluk (1955). Despite an extensive search we were unable to find these glands in the large intestine in any of the cases studied, or in the terminal ileum affected by ulcerative colitis.

Severe ulceration with damage to the muscle coats and an acute inflammatory reaction in the muscle coats and subserosa were seen in specimens from patients with severe acute ulcerative colitis.

In chronic ulcerative colitis the inflammatory reaction was limited to the mucosa and submucosa, and only tiny perineural and perivascular collections

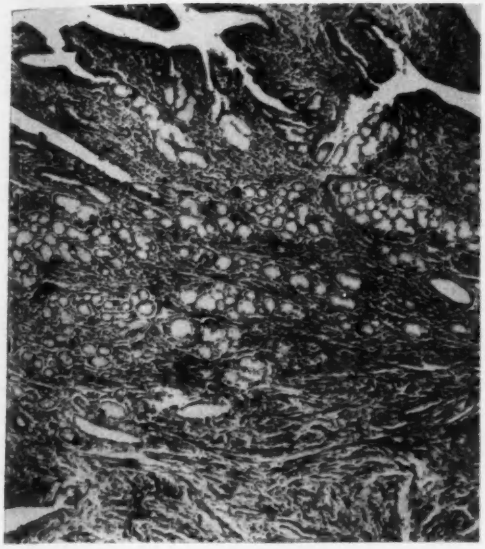

FIG. 9. *Brunner-type glands in the submucosa of the terminal ileum, from a patient with regional ileitis.* × 60.

of lymphocytes were seen in the muscle coats and subserosa. Mucosal tags and granulomatous polypi were found in 97 of the 157 specimens from patients with chronic ulcerative colitis. Crypt abscesses, mucosal ulceration, and some epithelial regeneration were present in all the specimens from patients with chronic active disease. Damage to the muscle coats was not seen and fibrosis, which was minimal, was limited to the superficial submucosa. Thickening of the bowel wall and narrowing of the intestinal lumen was invariably associated with a thickening of the muscle coats, and the myenteric plexus and ganglion cells were unusually prominent in these cases.

CARCINOMA Carcinomas were found in the specimens from 17 patients with ulcerative colitis. No carcinoma was found in the specimens from patients with primary Crohn's disease of the large intestine. Rectal carcinomas were found in specimens from two patients with regional ileitis. In the first case the rectal carcinoma was found five years after the surgical resection of the diseased terminal ileum and at the second operation there was no evidence of recurrent disease in the small intestine or of regional ileitis involving the large intestine. In the second case the patient presented with a rectal carcinoma and regional ileitis and both were treated by surgical resection. There was no evidence of regional ileitis involving the rectum, and the remainder of the large intestine appeared normal.

TABLE III

RELATIONSHIP BETWEEN LENGTH OF HISTORY AND DEVELOPMENT OF CARCINOMA IN DISEASED INTESTINE

Length of History (years)	*Number of Cases*			
	Regional Ileitis[1]	*Primary Crohn's Disease of Large Intestine*[1]	*Ulcerative Colitis*	
			Total	*With Carcinoma*
0-5	42	28	49	2
6-10	21	10	70	3
11-15	11	4	32	1
16-20	7	1	22	7
21-25	3	1	17	3
26-30	0	0	5	1
30 plus	2	1	5	0
Total	86	45	200	17

[1]No carcinoma was found in areas of regional ileitis or primary Crohn's disease of the large intestine.

The failure to demonstrate carcinoma in areas of regional ileitis or primary Crohn's disease of the large intestine may be related to their shorter length of history (Table III). Whilst the total incidence of carcinoma in patients with ulcerative colitis treated by all forms of surgery was 8·6%, the incidence in those with ulcerative colitis of more than 10 years' duration was 14·8%, and of more than 15 years' duration was 22·4%.

INCIDENCE OF PRIMARY CROHN'S DISEASE OF THE LARGE INTESTINE From January 1949 until the end of January 1960 surgical resections of a part or all of the large intestine were performed at the Gordon Hospital, London, on 200 patients with ulcerative colitis. In the same period and at the same hospital surgical resections of part or all of the large intestine were performed on 13 patients with primary Crohn's disease of the large intestine. The surgical incidence of primary Crohn's disease of the large intestine relative to ulcerative colitis was therefore roughly one in every 15 cases.

RECURRENCE AFTER SURGICAL RESECTION No recurrence was found in the small intestine after total colectomy for ulcerative colitis. Recurrence in the previously unaffected right side of the colon was found in two patients with ulcerative colitis treated by left hemicolectomy.

So far no recurrence has been found in the small intestine following the surgical treatment of primary Crohn's disease of the large intestine. Recurrence in previously unaffected segments of large intestine within a year of operation occurred in four patients treated by subtotal colectomy. In two patients the disease recurred in segments immediately proximal

to the primary lesion, and in two patients in segments immediately distal to the primary lesion.

Thirty-two of the 86 patients with regional ileitis developed a recurrence in unaffected segments of the small or large intestine after surgical resection. A second resection was performed on 28 of these patients. In the four remaining patients recurrence was indicated by a recurrence of symptoms and radiological evidence of disease. Recurrent disease affected the ileum and jejunum in one case, the ileum in 23 cases, and the ileum and large intestine in eight cases. In 13 cases disease recurred within two years, in 22 cases within five years, in 31 cases within 10 years, and in all the cases within 12 years.

DISCUSSION

Because these studies are based solely on surgical material they necessarily give a biased picture of the diseases investigated since they ignore all the patients with the milder forms of these diseases who did not require any surgical treatment. This is particularly important when complications such as fistulae or carcinoma are considered, conditions which necessarily bring the patient to the surgical clinic. However, the careful examination of resected specimens provides the most reliable method so far available of distinguishing cases of primary Crohn's disease of the large intestine from that of ulcerative colitis.

It may be argued that our histological criteria for the diagnosis of primary Crohn's disease of the large intestine are too strict, and that we have excluded cases which others might regard as examples of this condition. In our present state of knowledge we believe this to be a wise procedure. Nothing is gained and much may be lost by trying to classify doubtful specimens as atypical cases of ulcerative colitis or atypical cases of Crohn's disease of the large intestine.

Despite these and other limitations the results show that it is worth distinguishing between ulcerative colitis, primary Crohn's disease of the large intestine, and regional ileitis with large intestinal involvement.

CLINICAL FEATURES In this study regional ileitis was found to be commoner in men than in women, and primary Crohn's disease of the large intestine to be commoner in women than in men. Primary Crohn's disease of the large intestine occurred in an older age group than regional ileitis or ulcerative colitis, and approximately one-quarter of the cases occurred in women over 60 years of age. In the series of 25 cases reported by Lockhart-Mummery and Morson their seven cases of regional ileitis with large intestinal involvement all occurred in men, whose ages ranged from 17 to 49 years with a mean of 27 years. Of their 18 cases of primary Crohn's disease of the large intestine, 10 occurred in men and eight in women, their ages ranging from 34 to 72 years with a mean of 54 years.

Patients with primary Crohn's disease of the large intestine frequently present with segmental colitis. When only a short segment of the large intestine is affected the lesion can mimic carcinoma (Moynihan, 1907; Robson, 1908), and the frequent occurrence of primary Crohn's disease of the large intestine in patients over 50 years of age adds to the difficulty in separating the two diseases clinically. Similarly when Crohn's disease is limited to the sigmoid colon and the patient is over 50 years of age a diagnosis of diverticulitis may be made. This happened twice in our series and subtotal colectomies were performed. Examination of the operation specimens, however, showed Crohn's disease and no diverticulum could be found.

A gradual onset and a continuous course were common findings in patients with primary Crohn's disease of the large intestine and this probably accounts for their short clinical histories before surgical resections were undertaken. It is also possible that the Crohn's disease might have been latent in the colon for several years before its presence became clinically manifest. Periods of complete freedom from disease lasting a month or more, found in more than half the patients with ulcerative colitis, were uncommon in patients with primary Crohn's disease of the large intestine. Diarrhoea and melaena, characteristic features of ulcerative colitis, occurred less frequently and were less severe in most of the patients with primary Crohn's disease of the large intestine. These clinical observations tied up with the lesser degree of mucosal ulceration and the more frequent finding of normal haemoglobin levels in patients with Crohn's disease of the large intestine than in patients with ulcerative colitis.

FISTULAE Perianal and rectovaginal fistulae were found more frequently in patients with primary Crohn's disease of the large intestine than in patients with regional ileitis or ulcerative colitis. Similar findings have been reported by Lockhart-Mummery and Morson. In their series anal fistulae were found in 42·8% of the cases of regional ileitis with large intestinal involvement, and in 77·8% of the cases of primary Crohn's disease of the large intestine. The finding of multiple fistulae, indolent anal lesions, and recurrence after surgical treatment suggests a diagnosis of Crohn's disease rather than ulcerative colitis. All material removed at operations on anal fistulae should be sent to the laboratory for examination. The finding of non-caseating tubercles with Langhans

type giant cells in this material may then draw attention to a previously unsuspected case of regional ileitis or primary Crohn's disease of the large intestine (Morson and Lockhart-Mummery, 1959).

RECTAL DISEASE Difficulty may be experienced in separating the sigmoidoscopic appearances of ulcerative colitis and Crohn's disease. The finding of granular areas with intervening areas of normal or slightly oedematous mucosa suggests the diagnosis of Crohn's disease (Lockhart-Mummery and Morson, 1960). Rectal biopsy may help to establish the diagnosis of Crohn's disease when non-caseating tubercles with Langhans type giant cells are found. The majority of biopsies, however, fail to show these tubercles, and no great importance should be attached to a negative finding.

PATHOLOGY Although the combination of Crohn's disease and ulcerative colitis occurring in the same patient has been frequently reported in the American literature (Crohn and Yarnis, 1958; Colcock and Vansant, 1960), we have not seen any example of this combination. The few supposed examples which we have seen proved to be cases of ileostomy dysfunction after total proctocolectomy for ulcerative colitis (see Counsell, 1956), or cases of Crohn's disease of the large intestine in which few sections had been taken for histology and the presence of non-caseating tubercles in the intestinal wall or regional lymph nodes had been missed.

Finding Brunner-type glands in a tuberculous ulcer of the large intestine was first reported by Nicholson in 1923, and in 1951 Liber reported finding them in the terminal ileum of four patients with regional ileitis. We did not find these glands in the large intestine in any of the cases studied, and found them only in the terminal ileum affected by regional ileitis. Their partial staining reaction with indulin and mucicarmine and their occurrence only in areas of epithelial regeneration suggests a form of metaplasia developing in the healing phase of regional ileitis. Kawel and Tesluk (1955), however, reported a high incidence of recurrent disease after the surgical treatment of regional ileitis when these glands were present in the terminal ileum. In our series the recurrence rate was the same whether these glands were present or not. Similar findings have been reported by Antonius, Gump, Lattes, and Lepore (1960).

It is often stated that the histological appearances of the non-caseating nodules of epithelioid histiocytes found in patients with Crohn's disease are similar to those found in sarcoidosis. But a critical comparison of the lesions found in lymph nodes from patients with these diseases reveals a number of distinguishing features. In sarcoidosis, the lymph nodes are usually considerably enlarged and many non-caseating nodules of epithelioid cells are found inside them. In Crohn's disease, the nodules are found most frequently in normal-sized nodes and only a few nodules can be seen. In sarcoid, the nodules show a monotonous uniformity of size, shape, and stage of development. In Crohn's disease, the nodules vary considerably in size, shape, and degree of maturity, and are usually smaller than those seen in sarcoid. In sarcoid the nodules are cellular and compact. In Crohn's disease the epithelioid cells are more loosely arranged, and the cell margins are frequently separated by oedema. The multinucleated giant cells of sarcoidosis often contain more than 30 nuclei, whilst those in Crohn's disease seldom contain more than 16 and appear to be formed by the fusion of endothelial cells lining affected lymphatic capillaries.

It is not generally appreciated that sarcoid-like lesions can be found in the wall of the intestine close to carcinomas and villous papillomas (Dawson, 1961), and that similar lesions may be found in the regional lymph nodes draining carcinomas from many different parts of the body (Gresham and Ackerley, 1958). It is possible that some of the so-called cases of carcinoma with regional ileitis reported in the literature are of this type.

DEVELOPMENT OF CARCINOMA So far no definite tendency to malignant change has been established in patients suffering from Crohn's disease (Nevin, 1961). Carcinoma of the large intestine is, however, a well-recognized complication of ulcerative colitis, and its incidence appears to be related to the duration of the colitis rather than to the age at onset of the disease (Counsell and Dukes, 1952; Dawson and Pryse-Davies, 1959). The fact that no carcinoma occurred in areas of regional ileitis or primary Crohn's disease of the large intestine in the present series may be related to the finding that these patients came to surgery earlier than the patients with ulcerative colitis. It may also be related to the degree of mucosal ulceration, epithelial proliferation, and polyp formation being less marked in regional ileitis and primary Crohn's disease of the large intestine than in ulcerative colitis.

PROGNOSIS It is not the purpose of this communication to evaluate the different methods of treatment employed but we have noticed that several patients with primary Crohn's disease of the large intestine who had failed to obtain any adequate measure of relief by medical treatment were quickly restored to normal health by surgical treatment and have continued to enjoy a prolonged period of freedom from disease. Unnecessary delay in the surgical treatment of segmental colitis can result in rectal involvement,

the formation of perianal and rectovaginal fistulae, and the subsequent failure of ileorectal anastomosis. The high incidence of recurrent disease after surgical treatment found in patients with regional ileitis has not been found in patients with Crohn's disease arising primarily in the large intestine. It is too soon to judge whether primary Crohn's disease of the large intestine has a better prognosis than regional ileitis but the results of surgical resection are so far most encouraging.

SUMMARY

This study is based on 45 patients suffering from a non-specific chronic inflammatory disorder confined entirely to the large intestine. The pre-operative clinical diagnoses were idiopathic ulcerative colitis, diverticulitis, or carcinoma. Examination of the specimens removed at operation showed many of the characteristic pathological features of regional ileitis. For the sake of convenience these cases have been called primary Crohn's disease of the large intestine. The clinical and pathological features of these 45 cases are compared with 86 cases of regional ileitis and 200 cases of ulcerative colitis.

Compared with regional ileitis the disease occurred in an older age group, had a different sex distribution and a higher incidence of perianal and rectovaginal fistulae. A high incidence of recurrent disease following surgical treatment, found in the patients with regional ileitis, was not found in the patients with primary Crohn's disease of the large intestine.

The incidence of the disease relative to ulcerative colitis was approximately 6%. The disease had the same sex distribution as ulcerative colitis but occurred in an older age group. The disease usually had a gradual onset and continuous course, and periods of freedom lasting a month or more were uncommon. The patients came to surgery much earlier than patients with ulcerative colitis. Diarrhoea, melaena, and mucosal ulceration were less marked, and a normal pre-operative haemoglobin level was found in over 40% of the patients. Perianal and rectovaginal fistulae, found in two-thirds of the patients, were frequently multiple, indolent, and tended to recur after surgical treatment. The incidence of skip lesions and segmental colitis was much higher than that found in ulcerative colitis. The mucosal ulcers were usually elongated and fissured, and the intervening oedematous mucosa had a cobblestone appearance. The widespread ragged superficial ulceration, pseudo-polypi, dilated bowel and perforations, found in some cases of ulcerative colitis, were not seen in primary Crohn's disease of the large intestine.

Carcinomas of the large intestine were found in specimens from 17 patients with ulcerative colitis. No carcinoma was found in regional ileitis or primary Crohn's disease of the large intestine. This may be related to a shorter clinical history and a lesser degree of mucosal ulceration, epithelial proliferation, and polyp formation.

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REFERENCES

Antonius, J. I., Gump, F. E., Lattes, R., and Lepore, M. (1960). A study of certain microscopic features in regional enteritis, and their possible prognostic significance. *Gastroenterology*, **38**, 889-905.

Aylett, S. O. (1960). Diffuse ulcerative colitis and its treatment by ileo-rectal anastomosis. *Ann. roy. Coll. Surg. Engl.*, **27**, 260-284.

Braun, H. (1909). Über entzündliche Geschwülste am Darm. *Dtsch. Z. Chir.*, **100**, 1-12.

Brooke, B. N. (1959). Granulomatous diseases of the intestine. *Lancet*, **2**, 745-749.

Brown, P. W., Bargen, J. A., and Weber, H. M. (1934). Chronic inflammatory lesions of the small intestine (regional enteritis). *Amer. J. dig. Dis.*, **1**, 426-431.

Castleman, B. (1944). Case records of the Massachusetts General Hospital. Case 30172. *New Engl. J. Med.*, **230**, 526-529.

Colcock, B. P., and Vansant, J. H. (1960). Surgical treatment of regional enteritis. *New Engl. J. Med.*, **262**, 435-439.

Colp, R. (1934). A case of nonspecific granuloma of the terminal ileum and the cecum. *Surg. Clin. N. Amer.*, **14**, 443-449.

Counsell, B. (1956). Lesions of the ileum associated with ulcerative colitis. *Brit. J. Surg.*, **44**, 276-290.

——, and Dukes, C. E. (1952). The association of chronic ulcerative colitis and carcinoma of the rectum and colon. *Ibid.*, **39**, 485-495.

Crohn, B. B., Ginzburg, L., and Oppenheimer, G. D. (1932). Regional ileitis. A pathologic and clinical entity. *J. Amer. med. Ass.*, **99**, 1323-1329.

——, and Rosenak, B. D. (1936). A combined form of ileitis and colitis. *Ibid.*, **106**, 1-7.

——, and Yarnis, H. (1958). *Regional Ileitis*, 2nd ed. Grune and Stratton, New York and London.

Dalziel, T. K. (1913). Chronic interstitial enteritis. *Brit. med. J.*, **2**, 1068-1070.

Dawson, I. M. P. (1961). Villous papilloma of the rectum with giant cell systems. *Proc. roy. Soc. Med.*, **54**, 723.

——, and Pryse-Davies, J. (1959). The development of carcinoma of the large intestine in ulcerative colitis. *Brit. J. Surg.*, **47**, 113-128.

Donchess, J. C., and Warren, S. (1934). Chronic cicatrizing enteritis with involvement of the cecum and the colon. *Arch. Path. (Chicago)*, **18**, 22-29.

Dukes, C. E. (1954). The surgical pathology of ulcerative colitis. *Ann. roy. Coll. Surg. Engl.*, **14**, 389-400.
—— (1959). Personal communication.
——, and Lockhart-Mummery, H. E. (1957). Practical points in the pathology and surgical treatment of ulcerative colitis. *Brit. J. Surg.*, **45**, 25-36.
Goldgraber, M. B., Kirsner, J. B., and Palmer, W. L. (1959). The histopathology of chronic ulcerative colitis and its pathogenic implications. Proceedings of the World Congress of Gastroenterology, Washington, D.C., 1958. Vol. 2, pp. 935-943. Williams and Wilkins, Baltimore.
Goligher, J. C. (1961). Surgical treatment of ulcerative colitis. *Brit. med. J.*, **1**, 151-154.
Gresham, G. A., and Ackerley, A. G. (1958). Giant cell granulomata in regional lymph nodes of carcinoma. *J. clin. Path.*, **11**, 244-250.
Hadfield, G. (1939). The primary histological lesion of regional ileitis. *Lancet*, **2**, 773-775.
Harris, F. I., Bell, G. H., and Brunn, H. (1933). Chronic cicatrizing enteritis; regional ileitis (Crohn). A new surgical entity. *Surg. Gynec. Obstet.*, **57**, 637-645.
James, T. G. I. (1938). Chronic regional colitis. *Brit. J. Surg.*, **25**, 511-516.
Kawel, C. A., Jr., and Tesluk, H. (1955). Brunner-type glands in regional enteritis. *Gastroenterology*, **28**, 810-820.
Läwen, A. (1914). Über Appendicitis fibroplastica. *Dtsch. Z. Chir.*, **129**, 221-241.
Liber, A. F. (1951). Aberrant pyloric glands in regional enteritis. *A.M.A. Arch Path.*, **51**, 205-212.
Lockhart-Mummery, H. E., and Morson, B. C. (1960). Crohn's disease (regional enteritis) of the large intestine and its distinction from ulcerative colitis. *Gut*, **1**, 87-105.
Lumb, G. (1951). Cicatrizing enterocolitis. *Brit. J. Surg.*, **39**, 233-243.
——, and Protheroe, R. H. B. (1958). Ulcerative colitis. A pathologic study of 152 surgical specimens. *Gastroenterology*, **34**, 381-407.
Morson, B. C., and Lockhart-Mummery, H. E. (1959). Anal lesions in Crohn's disease. *Lancet*, **2**, 1122-1123.
Moschcowitz, E., and Wilensky, A. O. (1923). Non-specific granulomata of the intestine. *Amer. J. med. Sci.*, **166**, 48-66.
Moynihan, B. G. A. (1907). The mimicry of malignant disease in the large intestine. *Edinb. med. J.*, n.s. **21**, 228-236.
Neuman, H. W., Bargen, J. A., and Judd, E. S. (1954). A clinical study of two hundred and one cases of regional (segmental) colitis. *Surg. Gynec. Obstet.*, **99**, 563-571.
Nevin, R. W. (1961). A review of granulomata of the large intestine. *Proc. roy. Soc. Med.*, **54**, 137-142.
Nicholson, G. W. (1923). Heteromorphoses (metaplasia) of the alimentary tract. *J. Path. Bact.*, **26**, 399-417.
Rappaport, H., Burgoyne, F. H., and Smetana, H. F. (1951). The pathology of regional enteritis. *Milit. Surg.*, **109**, 463-502.
Robson, A. W. M. (1908). An address on some abdominal tumours simulating malignant disease, and their treatment. *Brit. med. J.*, **1**, 425-428.
Tietze, A. (1920). Über entzündliche Dickdarmgeschwülste. *Ergebn. Chir. Orthop.*, **12**, 211-273.
Warren, S., and Sommers, S. C. (1948). Cicatrizing enteritis (regional ileitis) as pathologic entity: analysis of 120 cases. *Amer. J. Path.*, **24**, 475-501.
——, —— (1954). Pathology of regional ileitis and ulcerative colitis. *J. Amer. med. Ass.*, **154**, 189-193.
Watkinson, G., Thompson, H., and Goligher, J. C. (1960). Right-sided or segmental ulcerative colitis. *Brit. J. Surg.*, **47**, 337-351.
Wells, C. (1952). Ulcerative colitis and Crohn's disease. *Ann. roy. Coll. Surg. Engl.*, **11**, 105-120.

Gut, 1961, **2**, 202

Difficulties in the diagnosis and management of unsuspected tuberculous enteritis and colitis

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SYNOPSIS Five patients with tuberculous enteritis and one with possible tuberculous colitis are described, all of whom presented difficult problems of diagnosis and management. Chest radiographs were normal in three cases, and radiology of the bowel was misleading in five. Two developed intestinal malabsorption with megaloblastic anaemia. The finding of tubercle bacilli in the faeces presented an important diagnostic problem in one of the cases with a normal chest radiograph. All the patients required both medical and surgical treatment, and the outcome in five has been very good and relatively satisfactory in the sixth. The last case particularly illustrates the difficulty of ultimate diagnosis in some patients with granulomatous or ulcerative entero-colitis.

Tuberculous enteritis and colitis are common problems in advanced pulmonary tuberculosis. There are also some abdominal conditions, such as dyspepsia, diarrhoea, and fistula-in-ano, which are well-known modes of presentation of pulmonary tuberculosis. There are, however, two groups of patients with tuberculous entero-colitis who present more difficult diagnostic problems. First, there are those patients whose abdominal condition is secondary to unsuspected pulmonary tuberculosis and who may have extensive investigations and treatment, including surgical operation, before the diagnosis is considered. Secondly, there is a rare group of patients with tuberculous enteritis or colitis without radiological evidence of pulmonary tuberculosis. The six patients described in this paper, all of whom were seen in a general hospital, illustrate the difficulties of diagnosis and management presented by such cases.

CASE 1 P.C., a housewife aged 34, began to have attacks of vomiting in 1947. Despite two barium meals and appendicectomy no cause was found until in April 1953 an episode of severe vomiting raised the possibility of intestinal obstruction. At laparotomy 15 stenosed areas were found, each about 1½ in. in length, scattered throughout the jejunum and ileum. There was also a thickened area in the caecum and ascending colon. The gross appearances were those of Crohn's disease. Histological examination of a nodule from the intestinal wall showed areas of endothelioid overgrowth with giant cells and minute areas of necrosis. A gland removed from the mesentery showed areas of caseation, and Ziehl-Neelsen staining showed small numbers of acid- and alcohol-fast mycobacteria (Professor R. W. Scarff).

In July 1953 she was admitted to the Middlesex Hospital under the care of Mr. R. Vaughan Hudson. Her only complaints were of epigastric and central abdominal pain after food, occasional vomiting, and amenorrhoea. Her weight was 7 st. 2½ lb. and temperature 99·4°F. The abdomen was slightly distended with diffuse guarding but no rigidity and no enlarged organs or abnormal masses were palpable. A radiograph of the chest showed infiltration in both upper zones and in the right midzone. Tomography showed a small cavity in the left upper zone, posteriorly. Examinations of one specimen of faeces and three specimens of gastric washings for tubercle bacilli by direct microscopy and culture were negative. The E.S.R. was 52 mm. in one hour (Wintrobe). The Hb concentration was 10·2 g. (Table I). A barium meal showed no abnormality in the oesophagus, stomach, or small bowel. The Mantoux reaction was positive at 1 in 100 (old tuberculin).

She was treated with bed rest, a high-calorie diet, and antituberculous chemotherapy, streptomycin 1 g. intramuscularly daily and isoniazid 200 mg. by mouth. During the next two months she improved considerably. Vomiting ceased and the abdominal pain decreased in severity; she became afebrile; her weight increased by 14 lb.; the E.S.R. (Westergren) was 5 mm. in one hour. The abdominal guarding, tenderness, and resistance, however, persisted. In January 1954 abdominal pain and vomiting returned. Although her general condition had improved, the abdominal signs had persisted and an indefinite mass was palpable in the right iliac fossa. Tubercle bacilli were not recovered from gastric washings or from the faeces. The cavity in the left lung was slightly smaller. A barium enema showed that the caecum was shrunken and deformed and the terminal ileum was dilated. A barium meal showed an apparent fistula between the duodenojejunal flexure and other loops of small gut. Multiple areas of stenosis and dilatation were seen in the small gut.

In view of the evidence of continuing intestinal obstruction, Mr. Vaughan Hudson performed a laparotomy in April 1954. Multiple strictures of the small bowel

TABLE I

	1953	*1954*				*1956*		*1957*
	July	*April*	*June*	*July*	*December*	*August*	*October*	*March*
Hb (%)	71	92	75	65	82	71	90	82
(g./100 ml.)	10·2	13·3	10·9	9·4	11·9	10·3	13·1	11·9
R.B.C. (m./c.mm.)	4·3	—	—	—	—	2·8	3·9	—
P.C.V. (%)	33	40	—	31	—	31	38	36·5
M.C.V. (c.μ.)	77	—	—	—	—	109	97	85
M.C.H.C. (%)	31	—	—	30	—	33	34	32·5
Total white cells	3,900	5,400	—	—	—	—	5,200	—
Neutrophils (%)	76	76	—	—	—	—	61	—
Lymphocytes (%)	11	17	—	—	—		31	—
Monocytes (%)	10	2	—	—	—	—	4	—
Eosinophils (%)	3	3	—	—	—	—	3	—
E.S.R. (mm./hr.)	52 (Wintrobe)	5 (Westergren)	—	—	—	—	3 (Westergren)	—
Serum								
Iron (μg./100 ml.)	—	—	—	—	—	—	120	—
B_{12} (μμg./ml.)	—	—	—	—	—	—	45	—
Folic acid absorption (Girdwood)	—	—	—	—	—	—	Normal	—
Marrow appearance	—	—	—	—	—	—	Partially treated megaloblastic	—
Plasma								
Albumin (g./100 ml.)	3·5	—	2·9	—	—	—	4·0	—
Globulin (g./100 ml.)	1·9	—	2·5	—	—	—	2·4	—
Treatment during immediately preceding few weeks	—	—	6 wk. post-operative	10 wk. post-operative	—	—	Folic acid	Folic acid B_{12}

and ascending colon were found. No tubercles were visible on the peritoneum nor was there any exudate, adhesion, or fistula. The first three strictures were bypassed by a series of side-to-side anastomoses leaving the affected bowel *in situ*. The distal ileum was extensively diseased and was removed, together with the ascending colon. Approximately 11 ft. of small intestine was left. The resected specimen measured 4 ft. Histological examination showed multiple areas of dense fibrosis and some areas of non-specific inflammation with ulceration of the mucosa but no histological indication of active tuberculosis.

In view of the histological evidence of a fibrosing granulomatous condition it was decided to treat her with cortisone together with streptomycin in the hope of preventing further stricture formation. She was given six weeks' treatment with cortisone 100 mg. daily by mouth together with streptomycin 1 g. intramuscularly and isoniazid 200 mg. orally. There was little clinical change directly attributable to this treatment but the steady improvement in her general condition continued, and in September 1954 she was discharged to convalescence.

In 1956 she developed a megaloblastic anaemia which responded to vitamin B_{12} (Table I) and a mild peripheral neuropathy which responded to vitamin B complex. When last seen in 1961, she was very well.

CASE 2 C.H., a soldier aged 28, began in 1939 to have attacks of epigastric pain, vomiting, and diarrhoea. The appendix was removed without benefit. His symptoms recurred intermittently for the next eight years and he was investigated at four hospitals. In February 1948 a barium meal showed a filling defect in the ileo-caecal region suggestive of Crohn's disease. At laparotomy four areas of thickening of the small intestine were found between the upper jejunum and the lower ileum. Tubercles were found in the mesentery and histological examination of one showed a small area of caseation but no bacilli. A chest radiograph after this operation showed infiltration in both upper zones, more marked on the right, where there appeared to be some calcification. Tubercle bacilli were recovered from gastric washings by guinea-pig inoculation, but not from the faeces. The Mantoux reaction was positive to second strength purified protein derivative (P.P.D.). Plasma protein concentrations were normal. A barium enema showed the caecum to be high in position but no other abnormality. A further barium meal detected no lesion above the lower ileum.

In January 1949 he was transferred to the Middlesex Hospital under the care of Mr. D. H. Patey. At this time he was having infrequent attacks of abdominal pain and diarrhoea. His general condition was good, his weight normal, and he was afebrile. The abdomen was slightly tumid, there was considerable guarding with localized tenderness and an ill-defined mass to the right of the umbilicus. A radiograph of the chest showed infiltration in both upper zones and the right midzone. A barium meal detected no abnormality in the jejunum or upper ileum; the extreme terminal ileum was dilated and showed absence of peristalsis. The caecum and ascending colon were narrowed, the mucosal pattern was destroyed, and haustration lost. The blood count and E.S.R. were normal. At laparotomy in January 1949, two strictures were found, one in the upper jejunum and the other 3 ft. from the ileocaecal junction. In addition there was induration and infiltration of the caecum and ascending colon. Mesenteric glands were enlarged. The strictures were bypassed by a side-to-side anatomosis and a right hemicolectomy performed, the terminal foot of ileum being removed. Histological examination of the thickened gut walls showed granulomatous giant cell systems with overlying ulceration and acute inflammation. The lymph

glands contained giant cell systems with small areas of caseation (Dr. J. B. Walters). Tubercle bacilli were not sought.

Ten days after operation a right pleural effusion containing straw-coloured fluid was found. A radiograph of the chest showed in addition to the pleural effusion some spread of the lesions in the upper zones as well as some basal collapse. Tubercle bacilli were cultured from a gastric washing.

The patient was treated with bed-rest and chemotherapy and later put on a sanatorium régime. Since November 1949 he has remained well. Chest radiographs showed progressive calcification of the lesions until 1953; since then they have remained stationary. His bowels are open once or twice daily and he has developed no anaemia or other evidence of malabsorption.

CASE 3 M.M., a tailor aged 35, was found in 1945 to have an abnormality in a radiograph of the chest. No further investigation was carried out. In 1946 he noticed a 'warm feeling' in the lower abdomen after urination associated with frequency. Urological investigation found nothing abnormal.

Early in 1947 he developed a feeling of pressure around the upper abdomen which was most noticeable when lying on the right side or after heavy meals and was reduced by avoiding food. In August 1947, while on board ship for South Africa, his bowels began to open three times a day but the stools were of normal character. In South Africa active pulmonary tuberculosis was diagnosed and he was treated with a sanatorium régime and pneumoperitoneum. Sputum and stomach washings and faeces did not produce tubercle bacilli on direct examination or guinea-pig inoculation. His general health improved but the abdominal symptoms persisted and in 1949 a barium meal and follow-through was said to be normal. In 1949 he returned to Britain and in 1951 was admitted to the Middlesex Hospital under the care of Dr. G. D. Hadley for investigation of the abdominal symptoms and loose stools. He had also lost some weight and vomited occasionally. He was afebrile and in good general condition. There were no abnormal signs in the chest. In the abdomen there was a firm mobile tender mass in the right iliac fossa.

A radiograph of the chest showed diffuse mottling in all lung fields, particularly in the upper zones. A barium enema showed the caecum to be stenosed and suggested that there were some strictures in the terminal ileum. Examination of three samples of sputum and three of faeces for acid-fast bacilli by smear and culture were negative. The E.S.R. was 34 mm. in one hour (Westergren) and there was a mild hypochromic anaemia of 83% (12·0 g./100 ml.). The Mantoux test was positive at 1 in 1,000.

The favoured diagnosis at this time was Crohn's disease with sarcoidosis of the lungs. This conclusion was based on the combination of a widespread infiltration in the lungs and a lesion in the bowel added to the failure to find tubercle bacilli in the sputum or faeces in 1947 and 1951.

In February 1951, Mr. C. J. B. Murray performed laparotomy. A large mass was found in the region of the caecum of which the gross appearance was suggestive of Crohn's disease. Within the last 3 ft. of the ileum there were thickened segments of gut, which in two places formed strictures. There was a further segment 6 ft. from the ileo-caecal valve. The mesentery contained firm, enlarged glands. A right hemicolectomy was performed with a side-to-side anastomosis. The most proximal lesion was excised and the ileum anastomosed end-to-end. The post-operative course was uneventful.

In addition to the operative findings the resected gut showed mucosal ulceration at the sites of the strictures. Section of the caecal wall showed tuberculous granulation tissue with many giant cell systems but little caseation. The ileum showed small peritoneal tubercles and there were small tubercles in the glands. The appearances were those of ileocaecal tuberculosis (Dr. A. D. Thomson). A culture taken from the caecal mass grew *M. tuberculosis*. The organism was also isolated by guinea-pig inoculation with extracts from the caecum and mesenteric glands.

The patient's abdominal discomfort disappeared and his general condition improved. His bowels were open once or twice daily, producing loose stools.

In August 1951, tomography suggested the presence of a small cavity at the apex of the right lung. Tubercle bacilli were not recovered from the sputum. As his general condition was satisfactory and the plain radiograph showed little change, he was kept under observation without antituberculous treatment. In February 1952 he developed lupus vulgaris on the legs which cleared up with Kromayer lamp therapy.

During 1953 radiographs of the chest showed that the lesion was progressing, and in September 1953 tubercle bacilli were found in three samples of sputum on direct examination and culture. He was treated with streptomycin, P.A.S., and isoniazid in various combinations until July 1954. His sputum became negative in January 1954.

When last contacted in November 1960, he was well and his chest radiographs had been unchanged for five years. Although his bowels were only open once or twice a day, the stools tended to be loose.

CASE 4 N.B., a housewife aged 52, was well until February 1951, when she noticed undue tiredness and loss of weight. In May, there was a gradual onset of diarrhoea, with three to five slimy, offensive, occasionally pale stools each day. In June 1951 she was found to have a hypochromic anaemia (Hb 9·3 g./100 ml.), a normal white count, and an E.S.R. of 57 mm. in one hour. A barium enema and meal showed no abnormality.

She was treated with iron by mouth without benefit and then with folic acid. Her condition, however, continued to deteriorate and in September 1951 oedema of the ankles appeared. She was admitted to the Middlesex Hospital under the care of Dr. H. L. Marriott in December 1951.

At the time of admission she weighed 5 stone (70 lb.) and, in addition to the symptoms noted above, she admitted to anorexia, exhaustion, night sweats, dyspnoea, and praecordial pain on exertion. She was found to be emaciated and to have an evening fever (99·4°F.). There was generalized brown pigmentation of the skin but not of the mucous membranes, Public and axillary hair was

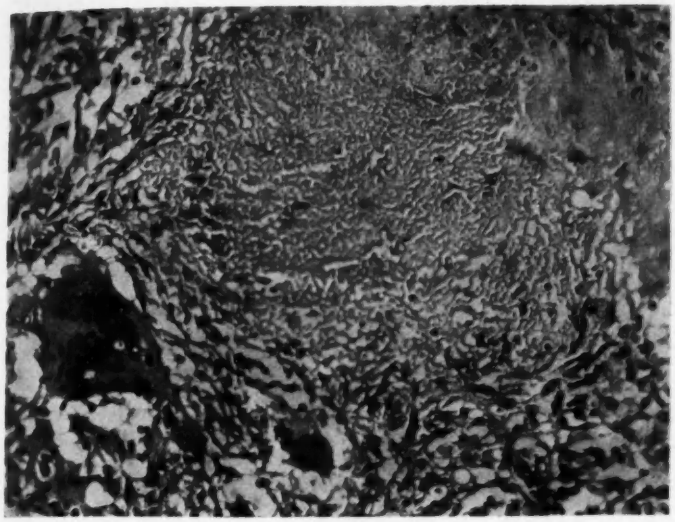

FIG 1. *Section from lymph node in Case 4 showing giant cells, epitheloid cells, and extensive necrosis* (× *230*).

lost. The feet and legs were oedematous. The blood pressure was 130/70 mm. Hg. There was no engorgement of the neck veins or clinical evidence of pulmonary congestion. No abnormality was found in the abdomen. The urine contained no excess of protein. The results of investigations performed on admission were as follows: Hb 9·4 g.; M.C.H.C. 27·5% (Table II), plasma protein concentration 5·2 g./100 ml. (albumin 2·4%, globulin 2·8%). A chest radiograph showed a calcified focus in the right lower zone and the right costophrenic angle was slightly obscured.

The immediate practical problem was to improve the malnutrition and anaemia. She was, therefore, treated with a diet of 5,000 calories and 120 g. protein per day, with iron and full vitamin supplements by intragastric drip. Her condition improved and she was then further investigated. The faeces contained no occult blood or excess of fat. The blood urea was normal. The serum alkaline phosphatase concentration was 15·6 units per 100 ml. (the serum calcium and phosphate concentrations were not measured). Sigmoidoscopy and barium enema were normal. The stomach secreted hydrochloric acid. The bone marrow showed generalized hyperplasia; the erythroid series were mainly normoblastic but the occasional appearance of a large 'intermediate' form suggested a commencing deficiency of the vitamin B group. (She was given 30 mg. folic acid daily for about three weeks after this report.) A barium meal showed an abnormal pattern in the ileum suggestive of regional ileitis.

Two weeks after admission she developed an irregular high fever without any fresh symptoms or physical signs. There was no leucocytosis and no response to penicillin. Regional ileitis seemed the most likely diagnosis.

Laparotomy by Mr. C. J. B. Murray in January 1952 showed that the terminal 3 ft. of ileum and caecum was thickened and in places adherent to each other. The lumen was narrowed. The affected bowel was resected together with the ascending colon, and an ileo-transverse colostomy performed. The resected bowel had numerous tubercles on the serous coat and was greatly thickened with multiple strictures and large areas of mucosal ulceration. Histological examination showed that there were numerous giant cell systems with areas of caseation (Fig. 1). The appearances were those of ileo-caecal tuberculosis (Dr. A. D. Thomson).

The improvement in her general condition after operation was very satisfactory; the temperature became normal and the bowels were only open once or twice daily. She was discharged home and when seen in July 1952 she was very well and had gained 3 stone (42 lb.) in weight.

She remained well without any treatment until February 1954 when diarrhoea returned and she passed five or six liquid brown stools daily. She also became progressively tired and weak and in the summer of 1954 her ankles began to swell. She was re-admitted in September 1954 having lost 2½ stone (35 lb.) in the preceding six months.

On admission she was found to have a low-grade fever of 99 to 100°F. There was pitting oedema of the legs but no abnormal findings in the abdomen and no signs of peripheral neuropathy. The mucous membranes were pale. A blood count showed a macrocytic anaemia with a Hb concentration of 6·0 g./100 ml. (Table II) and a few megaloblasts in the peripheral blood. The sternal bone marrow showed intense megaloblastic erythropoesis. Findings in addition to those recorded in Table II were: Serum bilirubin 2·2 mg. per 100 ml.; a sample of faeces contained over 50% fat; there was no occult blood in the faeces and tubercle bacilli could not be cultured from them; a fat balance showed 92% absorption on an intake of 73 g. fat per day; most of the bones of the skeleton were abnormally radiolucent.

TABLE II

	1951		1954				1955	1958
	June	December	22 September	8 October	18 October	December	June	January
Hb (%)	64	65	38	63	72	73	75	76
(g./100 ml.)	9·3	9·4	5·5	9·1	10·4	10·4	10·9	11·0
R.B.C. (m./c.mm.)	—	—	1·35	3·6	3·5	3·8	4·2	4·2
P.C.V. (%)	—	34	17	34	34·5	35	35	38
M.C.V. (cμ)	—	—	126	94	98	92	93	88
M.C.H.C. (%)	Hypo-chromic	27·5	32·5	27	30	29	31	29
Total white cells	—	4,300	3,100	5,600	9,000	4,600	4,500	—
Neutrophils (%)	—	17	65	—	90	75	73	—
Lymphocytes (%)	—	18	28	—	4	17	24	—
Monocytes (%)	—	5	6	—	2	4	3	—
Eosinophils (%)	—	—	—	—	4	2	—	—
Serum								
Iron (μg./100 ml.)	—	—	80	30	178	—	42	—
B_{12} (μμg./ml.)	—	—	80	—	—	—	—	—
Marrow appearance	—	Intense normoblastic ? early megaloblastic	Megalo-blastic	—	—	—	—	—
Plasma								
Albumin (g./100 ml.)	—	2·4	3·1	—	—	—	4·4	—
Globulin (g./100 ml.)	—	2·8	2·0	—	—	—	2·7	—
Serum								
Ca (mg./100 ml.)	—	—	7·4	—	—	—	10·2	—
Phosphorous (mg./100 ml.)	—	—	3·3	—	—	—	2·9	—
Phosphatase (units)	—	15·6	2·3	—	—	—	—	—
Treatment during immediately preceding few weeks — Iron	—	Oral		Oral Parenteral	Parenteral Parenteral	Oral Parenteral	Parenteral Parenteral	Parenteral Parenteral
B_{12}	—	—	—					
Folic acid	—	Oral	—	—	—	Oral	Oral	Oral

After 1 mg. of vitamin B_{12} intramuscularly there was a reticulocytosis of 42%, the serum bilirubin level fell to 0·5 mg. per 100 ml., and the macrocytosis disappeared. The Hb concentration rose to 9·2 g./100 ml. and the M.C.V. fell to 94 c.μ. The M.C.H.C., however, fell to 27% and the serum iron concentration to 30 mg. per 100 ml. in spite of the fact that she was receiving oral iron. Despite intensive treatment with parenteral iron and vitamin B_{12}, folic acid by mouth, and a high-protein, low-fat diet the anaemia has never been fully corrected.

In June 1955 she had gained 1 stone (14 lb.) in weight and felt well in herself. Her bowels were open two to three times a day. Her only significant complaint was of cramp in the legs at night. There were no abnormal physical signs. Investigations showed that the plasma protein concentration and calcium concentration were now normal. A barium meal showed no abnormality in the remainder of the small intestine.

When last heard of in January 1958, she was leading a normal life. Her weight was 8 st. 10 lb. (130 lb.) and her bowels were acting normally.

CASE 5[1] N.C., a Cypriot cook aged 29, began to have attacks of pain in the right lower abdomen in May 1959. The pain usually came on after his evening meal, and sometimes spread to the left side. The pain had no other characteristic features and, apart from loss of 14 lb. weight, was unassociated with other symptoms. He was admitted to the Middlesex Hospital under the care of Dr. G. D. Hadley in August 1959, with an apparently severe attack of pain. On examination, his general condition was good and the temperature was normal. A mobile firm, smooth swelling, 2 in. × 1 in., was palpable in the right iliac fossa. The E.S.R. was 24 mm. in one hour (Westergren), the plasma globulin concentration was 3·7 g./100 ml. and the white cell count 2,900 per c.mm. (61% polymorphs, 25% lymphocytes, and 12% monocytes). Three out of five samples of faeces contained occult blood. A chest radiograph, a barium meal (with follow-through) and barium enema on two occasions were normal. He was transferred to the care of Mr. J. H. L. Ferguson and at laparotomy the mass was found to be composed of caseous lymph nodes in the ileo-caecal mesentery. The external appearance of the bowel was normal. The terminal ileum, caecum, and proximal half of the ascending colon were resected. The specimen was found to contain two small ulcers in the ileum and ascending colon. Both these ulcers and the nodes contained numerous tubercles, giant cells, caseation, and acid-alcohol fast bacilli (Dr. R. E. Cotton).

After operation, two samples of sputum were examined for tubercle bacilli; none was seen, but the culture from one of the samples was positive. Cultures of the urine were negative. He was treated with antituberculous chemotherapy, and a prolonged convalescence. In August 1960, he was well and a chest radiograph was still normal.

CASE 6 N.D., a housewife aged 26, was well until April 1953, when she developed mild diarrhoea followed by lassitude and a fever of up to 104°F. In June 1953 she was extensively investigated without a diagnosis being reached. She was treated with short courses of various antibiotics (including streptomycin) to none of which did she respond.

In August, she was transferred to the Middlesex Hospital under the care of Dr. H. L. Marriott. On

[1]This case is to be described in more detail by Ashken and Baron.

admission her weight was 5 stone (70 lb.), a loss of 2 stone (28 lb.) in seven months. Additional symptoms were amenorrhoea for three months and redness of the eyes.

She was very thin, pale, and quiet but mentally alert. She had a continuous irregular fever of 100 to 102°F. and a pulse rate of 120 to 140. There was a mild phlyctenular conjunctivitis. The only other physical signs were in the abdomen, which was slightly distended and the spleen tip was palpable; there did not appear to be any ascites. There were no masses or areas of tenderness. In view of the possible diagnosis of tuberculous peritonitis, paracentesis of the abdomen was attempted but no fluid was found.

She was extensively investigated for the usual causes of pyrexia without a diagnosis being reached until the faeces were specially examined for tubercle bacilli which were found on direct examination of two successive stools. The bacilli were cultured from one of these stools, and subsequently identified as being of human type.

The results of other relevant investigations performed at this time were as follows. A chest radiograph was normal. Hb concentration was 7·2 g./100 ml., red cells 3·1 m. per c.mm., M.C.V. 80 c.μ., M.C.H.C. 29%, white cells 7,500 per c.mm. (neutrophils 63%, lymphocytes 26%, monocytes 10%). A Mantoux test (1 in 1,000 O.T.) was positive; gastric washings contained no tubercle bacilli.

She was thought to be suffering either from miliary tuberculosis or tuberculous enteritis. Treatment with streptomycin and isoniazid was begun and she also received extensive measures, including high-calorie supplementary feeds and blood transfusion designed to improve her general condition.

At the end of October her condition was sufficiently improved to permit further investigation. A barium enema examination was technically difficult but revealed no abnormality in the colon or terminal few inches of the ileum. The appearances at a barium meal were normal in the upper jejunum but in the lower jejunum and upper ileum the mucosal pattern had coarsened and the barium had become clumped. A stricture appeared to be present in this region. The lower ileum was not satisfactorily visualized.

These findings suggested that there might be a localized tuberculous ileitis.

At laparotomy by Mr. C. J. B. Murray in November 1953, the small intestine was found to be normal; the caecum was thickened and the serous coat was studded with tubercles; there were enlarged glands in the mesentery. There was a similar but smaller area of disease in the transverse colon near the splenic flexure. A right hemicolectomy was performed and an ileo-transverse colostomy created, leaving the smaller diseased area *in situ*.

The resected specimen showed multiple shallow serpiginous ulcers with polypoid overgrowth and some fibrosis of the wall of the bowel. On microscopy the ulcers were found to have perforated the mucosa and in places the muscle of the colon. There was infiltration with round cells and occasional giant cells. The appearances were considered to be compatible with treated tuberculosis (see later).

The patient's immediate post-operative course was satisfactory and her subsequent progress was excellent. She became afebrile within one week, and in six weeks she regained her normal weight of 7 stone (a gain of 22 lb.). Her bowel action was normal and the blood haemoglobin concentration was 13·6 g./100 ml. However, she still had a tachycardia when out of bed, the stools contained occult blood, and the E.S.R. was 15 to 20 mm. in one hour (Westergren). She was therefore given antituberculous chemotherapy for a further two months.

She remained well until July 1954, when she had a recurrence of diarrhoea passing four or five bloody stools each day, fever, loss of weight, and abdominal pain. She was re-admitted to the Middlesex Hospital under the care of Dr. F. H. Scadding in September 1954. On admission her weight was 7 stone (98 lb.); she had a regular swinging fever of 99 to 102°F. In the left hypochondrium there was tenderness, associated with guarding, and an ill-defined mass. The Hb concentration on admission was 10·9 g./100 ml. with a normal M.C.V. and M.C.H.C.; the white cell count was normal. Tubercle bacilli were not found in the facees (five samples) either on direct examination or on culture. A chest radiograph was normal.

It appeared probable that the tuberculosis at the splenic flexure had become active again. Treatment with streptomycin, isoniazid, and full supportive measures was begun. There was, however, little evidence of response. Throughout the next six weeks her fever continued unchanged; she lost 21 lb. in weight and the Hb concentration repeatedly fell to less than 10 g./100 ml. in spite of blood transfusions. The diarrhoea diminished slightly but the stools continued to be watery and to contain fresh blood.

Sigmoidoscopy showed that the rectum was relatively normal so Mr. C. J. B. Murray again performed a laparotomy. There was gross inflammation of the colon beyond the anastomosis, most marked at the splenic flexure, with a similar smaller area of disease at the recto-sigmoid junction. The colon was removed and an ileo-rectal side-to-side anastomosis formed. The histology of the resected bowel was identical with that removed at the first operation.

The patient's immediate postoperative course was satisfactory and four days later she was afebrile. She subsequently improved steadily with bed rest and continued antituberculous chemotherapy. Three months after operation, she had gained 1 stone in weight. Her bowels were open three or four times daily, with an unformed but not watery stool containing no fresh or occult blood. The Hb concentration was 12·3 g./100 ml. and E.S.R. (Westergren) 8 mm. in one hour. A fat balance showed a faecal loss of 5·8 g. per day on an intake of 70 g. fat.

The improvement continued after discharge and in May 1955 she had returned to her normal weight of 7 stone (98 lb.). Her bowel action was unchanged. She was given oral treatment with isoniazid and P.A.S. until November 1955 and she remained well for three years and had a baby in July 1958. In February 1959, diarrhoea recurred and the patient lost weight and experienced increasing muscular weakness. On re-admission in April 1959, her weight was 5½ stone (77 lb.), pulse rate 120, blood pressure 85/60 mm. Hg, and she was very weak. There were no

localized physical signs. She was found to have mild anaemia (Hb 12·9 g.) mild hypokalaemia and hyponatraemia (serum potassium 3·2 mEq./l. and sodium 132 mEq./l.). The blood urea was 40 mg./100 ml. and the daily 17-ketosteroid excretion was 1·5 mg. These findings were attributed to her poor general condition and the diarrhoea. A barium enema and meal suggested the presence of ulcers in the terminal ileum and a developing stricture at the ileo-rectal anastomosis. No tubercle bacilli were recovered from three samples of faeces by direct examination or culture. In view of the previous history and the radiological findings, she was treated with streptomycin and isoniazid as well as many supportive measures including corticosteroids. In spite of these, her condition gravely deteriorated, and she had episodes of hypothermia, hypotension, and unexplained obstructive jaundice. Dr. Avery Jones suggested that regional enteritis was a more likely diagnosis than tuberculous enteritis. Sigmoidoscopy showed a friable granular mucosa resembling ulcerative colitis. Antituberculous chemotherapy was stopped. She improved and in July 1959 left hospital still taking prednisone by mouth. When last seen in July 1960, she was very well, her bowels were open five or six times a day, and her weight was 95 lb.

The histology of the bowel resected in 1953 and 1954 was reviewed and regional enteritis was preferred to the original diagnosis of treated tuberculosis because there was no convincing evidence of caseation, the giant cells were too variable in type, many of them being of the foreign body variety, and the granulomatous process was too vascular (Dr. A. D. Thomson).

The diagnosis is further discussed below.

DISCUSSION

These cases illustrate first the variability of the clinical picture, secondly the difficulties in diagnosis, and thirdly the good results of modern treatment.

It must be admitted in retrospect that only in Case 6 was the clinical picture so unusual that the full diagnosis of the site as well as of the nature of the condition could not have been suspected.

In the first and second cases, the pulmonary lesions were probably of long standing, and an earlier chest radiograph might have suggested the diagnosis. In the third case the long history and very diffuse pulmonary infiltration combined with failure to find tubercle bacilli led to a diagnosis of sarcoidosis being preferred to that of tuberculosis.

Kogan and Janowitz (1956) have described seven cases of intestinal tuberculosis which were operated on before the diagnosis was suspected, and in three of these chests radiographs after operation showed pulmonary tuberculosis.

Cases 4, 5, and 6, on the other hand, raise the general problem of diagnosing intestinal tuberculosis in the presence of a normal chest radiograph. An examination of the literature suggests that in adults this is an uncommon problem. Unfortunately, most of the early literature on this subject was before the recognition of regional ileitis, and the diagnosis of primary hyperplastic tuberculosis in such series must be suspect. However, the extensive review of Brown and Sampson (1930) shows that, even before regional ileitis was described as a separate entity, the problem was uncommon. Following the description of regional ileitis, American writers (Bockus, 1944; Crohn and Yarnis, 1940) came to regard 'primary' tuberculous entero-colitis as an extreme rarity. This view must not be allowed to prevent tuberculosis being considered clinically in patients with normal chest radiographs. Two of seven cases in a series based on the years 1936-56 described by Kogan and Janowitz had negative chest radiographs, and nine of 58 cases of intestinal tuberculosis seen at the Mayo Clinic between 1921 and 1946 (Hoon, Dockerty, and Pemberton, 1950) had no evidence of extra-enteric tuberculosis. Paustian and Bockus (1959) only managed to find eight reports recording 25 cases of tuberculous enteritis in the absence of active pulmonary disease in the American literature since 1940. Anand (1956) has described from India 50 cases of hyperplastic ileo-caecal tuberculosis, 39 of which had no pulmonary lesion (apparently as judged by radiographs and sputum examination). Tubercle bacilli of human type were isolated from 25 of these 39 cases.

To these must be added my Cases 4 and 5 (and possibly Case 6). Obviously, these reports raise the pathological problem of the nature of Crohn's disease and its relation to tuberculosis. This consideration is outside the scope of this paper, but it is important to consider the possibility of tuberculosis in individual cases because of the therapeutic implications.

The confirmation of the diagnosis of tuberculosis in patients with normal chest radiographs presents a difficult problem. It would appear worthwhile to examine the faeces for tubercle bacilli. It is ironical that, in the present series, they were only found in Case 6 in which the diagnosis remains doubtful. The irony is heightened by the fact that the finding of tubercle bacilli in the stools was the most persuasive factor behind the decision to operate on such an extremely ill patient, and may therefore have saved her life.

The chief method of diagnosis in intestinal tuberculosis, of course, must be by radiology. Experience with Cases 1, 2, 3, 5, and 6 was in this connexion unfortunate, but illustrates the difficulties of the method. In Kogan and Janowitz's series, there were five patients in whom adequate radiological and surgical examination of the bowel permit comparison. In four of these the radiological findings, while showing some abnormality, seriously underestimated or misinterpreted the true situation. The

one patient in whom the radiological findings were accurate was studied by the direct instillation of a small amount of barium into the duodenum.

There are three ways in which radiology may be misleading; first by failing to find any abnormality in the presence of fairly extensive disease; secondly, by finding abnormalities which are not due to disease; and thirdly, by misinterpretation of the nature of those lesions which are detected. Radiologists are much more aware of the difficulties than are clinicians, and would be greatly helped if the latter gave them clear indications when granulomatous lesions are suspected, so that they can use special techniques.

The number of cases is too small and dissimilar to justify general conclusions about treatment, but the course of the illness in Case I suggests that chemotherapy was of benefit. Surgery was not radical in this patient, and much of the credit for the eventual outcome may be attributable to chemotherapy. When the granulomatous process is very marked, as in Case 1, the combined use of steroids and antituberculous chemotherapy may be worthy of trial.

The development of a serious malabsorption syndrome without significant clinical evidence of extensive tuberculous enteritis in Case 4 is more surprising than its recurrence after extensive resection in this patient or its appearance in Case 1. The presence of megaloblastic anaemia as part of the malabsorption syndrome is probably only a reflection of the duration of the condition; in neither case did it appear to be due to a blind loop.

Case 6 remains somewhat of a mystery. When this series of patients was collected in 1958, she appeared to be an example of primary tuberculous colitis, a condition which does not seem to have been described. It now seems possible that she has an unusual form of regional enteritis, verging on ulcerative colitis. She has been retained in this series for four reasons; first, she illustrates the difficulty of making a certain diagnosis in this group of conditions, even when histology is available; secondly, she illustrates the limitations of routine radiological techniques in this type of case; thirdly, she presents the problem of interpreting the finding of tubercle bacilli in the faeces. The explanation of their presence is very difficult, because tubercle bacilli only appear in the faeces in sufficient numbers to be seen on direct examination either in open cavitating pulmonary tuberculosis or in ulcerative tuberculosis of the gut. Their presence as contaminants in sufficient numbers for detection is unknown (O'Grady and Thompson, personal communication). Finally, therefore, we have the fourth reason for retaining this case in the series; she may, in fact have had tuberculous colitis. The conflict between the histological and bacteriological evidence may be resolved by reference to the experience of Wig, Singh, Chitkara, and Gupta (1954), who described four patients in whom tuberculous enteritis was diagnosed on tissue removed at operation (in one case, tubercle bacilli were recovered). At second operations after chemotherapy, only the changes of a non-specific granuloma were found. Taylor (1945) also described the disappearance of caseation necrosis and tubercle bacilli in the later stages of tuberculous enteritis.

I am grateful to all the physicians and surgeons, both at the Middlesex and other hospitals, for permission to describe these patients and for their advice in the preparation of these histories. I am also grateful to all the pathologists and radiologists for discussing their reports with me. Dr. M. P. Spence kindly read the manuscript. The photomicrographs were prepared by Drs. R. E. Cotton, A. C. Thackray, and A. D. Thomson.

REFERENCES

Anand, S. S. (1956). Hypertrophic ileo-caecal tuberculosis in India with a record of fifty hemicolectomies. *Ann. roy. Coll. Surg. Engl.*, **19**, 205-222.

Bockus, H. L. (1944). *Gastro-enterology*, Vol. 2, p. 197. Saunders, Philadelphia.

Brown, L., and Sampson, H. L. (1930). *Intestinal Tuberculosis: Its Importance, Diagnosis and Treatment*, 2nd ed. Lea and Febiger, Philadelphia.

Crohn, B. B., and Yarnis, H. (1940). Primary ileocecal tuberculosis. *N.Y.St. J. Med.*, **40**, 158-166.

Hoon, J. R., Dockerty, M. B., and Pemberton, J. de J. (1950). Ileocecal tuberculosis including a comparison of this disease with nonspecific regional enterocolitis and noncaseous tuberculated enterocolitis. *Int. Abstr. Surg.*, **91**, 417-440.

Kogan, E., and Janowitz, H. D. (1956). Intestinal tuberculosis: difficulties in diagnosis in the absence of florid pulmonary involvement. *J. Mt Sinai Hosp.*, **23**, 597-615.

Paustian, F. F., and Bockus, H. L. (1959). So-called primary ulcerohypertrophic ileocecal tuberculosis. *Amer. J. Med.*, **27**, 509-518.

Taylor, A. W. (1945). Chronic hypertrophic ileocaecal tuberculosis, and its relation to regional ileitis (Crohn's disease). *Brit. J. Surg.*, **33**, 178-181.

Wig, K. L., Singh, M. M., Chitkara, N. L., and Gupta, S. P. (1954). Observations on histological changes in cases of hyperplastic intestinal tuberculosis after treatment. *Indian J. Tuberc.*, **1**, 151-160.

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Recurrent swellings of the parotid gland

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SYNOPSIS This paper presents an analysis of 104 (43 men and 61 women) patients who had been investigated for recurrent swelling of the parotid gland. The investigation and causes of this condition are discussed.

Initially all patients had recurrent swellings which subsided completely between attacks, but in some, particularly in those with Sjögren's syndrome, the swellings ultimately became persistent. Table I shows distribution of cases in 10-year periods according to age and sex.

TABLE I

AGE AND SEX

	Age in Years 0-9	10-19	20-29	30-39	40-49	50-59	60+
Male	18	10	4	2	3	4	2
Female	8	4	5	12	8	14	10
Total	26	14	9	14	11	18	12

It will be seen that the largest group of cases occurs in the first two decades. There is a fall in the 20 year group followed by a high level after the age of 30 years. It is noticeable that whereas males predominate under the age of 20 females are greatly in excess after 30 years. Rose (1954), in his analysis of a rather larger group of mixed cases, also drew attention to the preponderance of females in the older group, as has Payne (1933, 1940). Jones (1953) records a slight excess of males over females in a group of 17 children.

The investigation has been undertaken on the basis of history, physical examination of the swellings and of Stenson's duct, examination of saliva obtained from the duct, and sialography. All these contribute to the diagnosis but for the moment the results of sialography only will be considered. For this a long No. 22G needle with solder attached 3-5 mm. from the distal end was used (Fig. 1). The solder can be wedged up against the duct orifice to prevent reflux. A watery solution of neohydriol is injected: Most normal duct systems will accommodate ½-1 ml. without rupture of the acini but when the main ducts are dilated 2 or 3 ml. may be required to obtain adequate filling. Sialograms fall into four main patterns: 1, Normal appearance; 2, dilatation of the terminal ducts or acini; 3, dilatation of the main and/or main branch ducts; 4, filling defects in main or branch ducts due to calculus with dilatation of ducts distal to obstruction. The presence of tumours may be indicated by displacement of the branch ducts, and parenchymatous swellings by separation of the ducts from one another.

Those cases falling into groups 2 and 3, according to age and sex, are analyzed in Table II.

TABLE II

ANALYSIS OF GROUPS 2 AND 3

	Age in Years 0-9	10-19	20-29	30-39	40-49	50-59	60+
	Terminal Dilatation						
Male	8	8	1	0	1	0	0
Female	4	3	1	3	1	2	1
Total	12	11	2	3	2	2	1
	Main Duct Dilatation						
Male	0	0	2	0	0	1	0
Female	0	0	2	2	3	9	6
Total	0	0	2	2	3	9	6

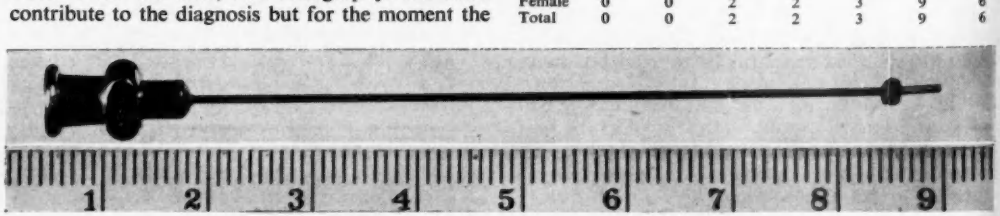

FIG. 1. *Needle employed for introduction of Neohydriol into Stenson's duct (scale in cm.).*

Terminal duct dilatation therefore occurs most frequently in the first two decades, with males predominating over females in the proportion of two to one. Main duct dilatation occurs very much more commonly in females and is rare under 40 years of age. Those with normal sialograms on the whole reflect the findings in the group with terminal duct dilatation, occurring most commonly before the age of 20 with a 4:1 excess of males over females. Those in whom sialography was not carried out clearly include cases from both the youthful group and the older female cases (Table III).

TABLE III

SIALOGRAM FINDINGS RELATED TO AGE AND SEX

	Age in Years 0-9	10-19	20-29	30-39	40-49	50-59	60-69
	Normal Sialograms						
Male	6	2	1	1	1	0	0
Female	1	1	3	4	1	1	0
Total	7	3	4	5	2	1	0
	Sialograms Not Done						
Male	4	0	0	0	0	0	0
Female	3	0	0	1	3	2	1
Total	7	0	0	1	3	2	1

There remain 14 patients with filling defects and localized distensions; these were all over 20, and included seven males and seven females. All had calculus formation and will be more fully discussed later.

For the purpose of this paper cases have been classified on clinical grounds under five main headings: 1, Infective; 2, Sjögren's syndrome; 3, calculus formation; 4, allergic; and 5, doubtful origin. It should be pointed out that infection of the duct system is not limited to group 1. Many cases of parotid swelling in Sjögren's syndrome are infected, and infection, possibly secondary, is present in many cases of calculus obstruction and may also occur in those with allergy.

INFECTIVE GROUP

These patients have periodic attacks of swelling of the parotid gland, usually for several days or weeks at a time with intervals of freedom. The gland is often tender, the duct orifice reddened, and purulent debris can sometimes be expressed from it. Organisms, including *Strep. viridans*, pneumococcus, *Staph. aureus*, haemolytic streptococci, and *Staph. albus*, have all been cultivated from the parotid secretions, but it is difficult to avoid contamination with mouth organisms, and cultures from saliva which do not contain pus should be accepted with caution. Patients frequently complain of an unpleasant taste in the mouth. In cases of doubt resolution after treatment with tetracycline or penicillin may provide confirmatory evidence of infection.

The group contains 39 patients: 21 were under the age of 20 and of these 15 were males. All those investigated by sialography had terminal duct dilatation (15) or normal ducts (4). Two also had irregular dilatation of one main duct. There were 16 cases over 30: 15 were females and one male. Ten had dilated main ducts, three terminal dilatation, and one a normal sialogram. In two cases sialograms were not obtained. Of those with dilatations of the terminal ducts, 12 had bilateral swellings, seven unilateral. Two of the latter showed terminal dilatation on the unaffected side as well as on the affected.

CASE 1 This boy, aged 10 years, is the only diabetic in the series. He has had recurrent swellings of the parotid glands for six years. The left side has been repeatedly affected but the right side on one occasion only. Pressure over the distended glands on the left side caused exudation of pus from Stenson's duct. *Strep. pneumoniae* was obtained on culture. Sialography showed that the terminal ducts were dilated on both sides (Fig. 2). A biopsy obtained from the left side (Fig. 3) shows dilated acini with fine interstitial fibrosis and patchy infiltration by chronic inflammatory cells. Polymorphs are present in some of the smaller ducts. The appearance is typical of a recurrent parotitis in an active phase.

The patients with main duct obstruction can be divided into those in whom obstruction was at the orifice of the duct (seven cases) and those in whom the obstruction occurred at some point in the course of the main duct distal from the orifice (six cases). Four in each group had bilateral swellings. Two patients with unilateral swellings showed obstruction at some distance from the duct orifice with an appearance very suggestive of calculus formation (filling defects in duct) but no confirmatory evidence was obtained. Terminal duct dilatation was present in two cases with dilatation of the main duct. In two

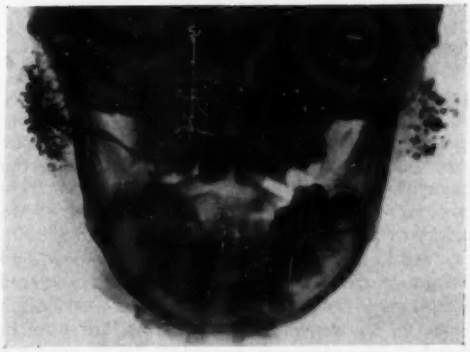

FIG. 2. *Case 1 showing bilateral terminal dilatation of ducts. Recurrent infection.*

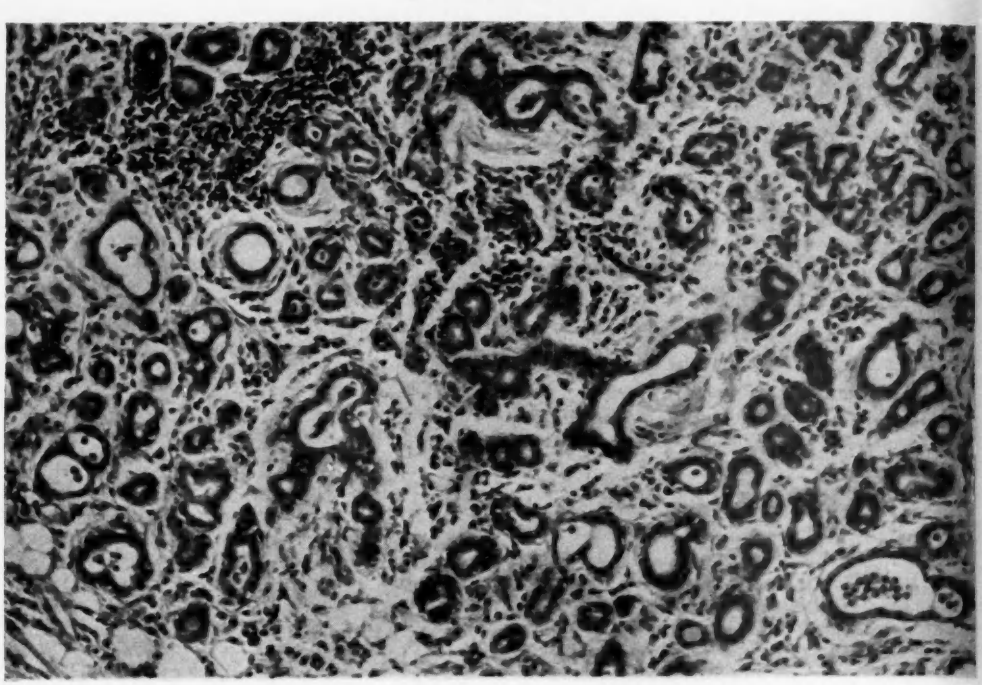

FIG. 3. *Case 1. Biopsy (haematoxylin and eosin × 200) showing changes of chronic infection.*

cases the swelling was first noticed shortly after extraction of teeth.

CASE 2 A woman aged 65 had had recurrent swellings of both parotid glands since she was 50 years of age. The swelling persisted for from two to seven days. Turbid purulent fluid was expressed from the duct and on culture *Strep. viridans* was grown. Both main ducts were dilated and biopsy of the glands showed a normal appearance apart from some atrophy and fat replacement (Fig. 4).

SJÖGREN'S SYNDROME

These patients presented with the characteristic history of dry mouth, dry, painful eyes, and in the majority widespread rheumatoid arthritis. Nineteen cases were investigated but six of these gave no history of parotid swelling at any time and are not included in this series. Some of them, however, had sialograms carried out and two showed scattered dilatations of terminal acini and one, dilated branch ducts. Of the remaining 13 patients, four were in their late 30s and nine were over 40. All were women except for one man of 68 years. The swellings were bilateral in 12 and fluctuated in size but rarely subsided completely. Sialograms were carried out in eight patients, two were normal, four had dilatations of the terminal ducts, and in two the main and branch ducts were dilated. Poor filling of branch ducts due to atrophic changes is characteristic of this condition. Two patients could not be investigated radiologically because thick, cheesy debris was constantly present in Stenson's duct from which only small quantities could be expressed. These two patients had persistent and extensive swelling of the parotid glands at the time of investigation. One patient (with main duct dilatation) had had an acute parotitis due to *Staph. aureus*, and surgical drainage was necessary on two occasions. The histology of the glands in this condition has been described by Cardell and Gurling (1954).

CASE 3 For four years this patient, a woman aged 54, had complained of multiple joint pains causing moderate disability. Three years previously the left parotid gland became swollen and red. Afterwards she noticed that her mouth became very dry. The swelling fluctuated but never entirely subsided. At a later date the right parotid gland became slightly swollen and marked kerato-conjunctivitis sicca was present.

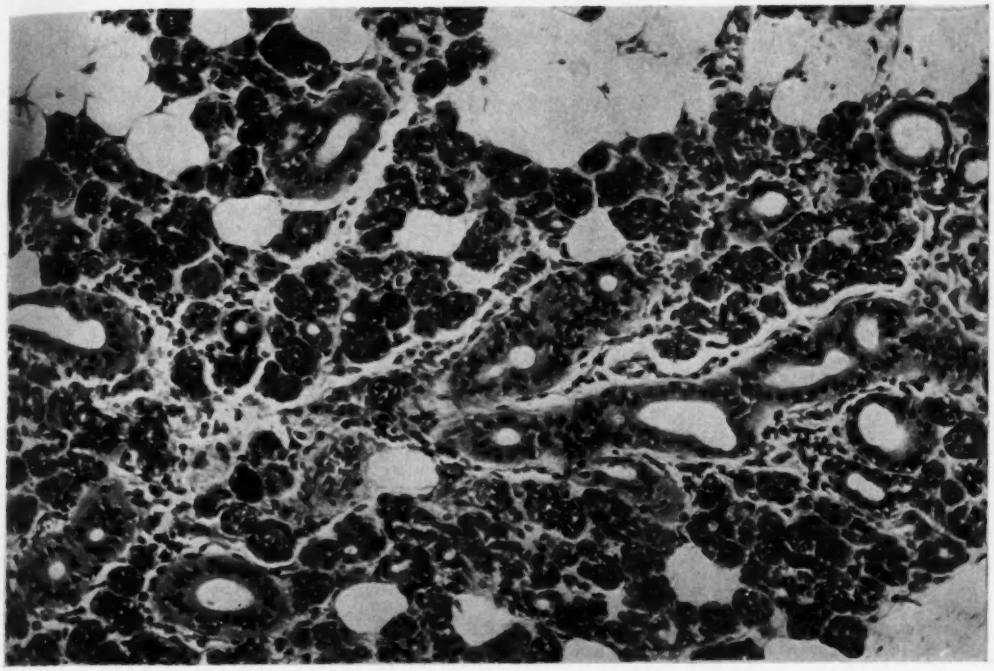

FIG. 4. *Case 2. Biopsy (haematoxylin and eosin × 200) showing minimal glandular atrophy and fat replacement.*

FIG. 5. *A sialogram of Case 3 showing terminal dilatation.*

A sialogram showed extensive dilatation of the terminal ducts and some irregular dilatation of the main duct (Fig. 5). Section on the left side confirmed the dilatation with evidence of chronic inflammation and residual glandular tissue undergoing hyalinization (Fig. 6).

CASE 4 A woman aged 36 first had symptoms of rheumatoid arthritis at the age of 23. These cleared up but returned after pregnancy at 29 and her condition has steadily deteriorated since then. At 28 she noticed swelling of the parotid glands on eating (for the past two years the swelling has been persistent). Treatment with corticotrophin caused the swelling to subside temporarily but no increase in secretion. For two years her mouth has been persistently dry and she has had a pricking sensation in the eyes. A sialogram showed dilatation of the terminal ducts. The branches of Stenson's duct were characteristically scanty due to atrophy of glandular tissue (Fig. 7). Section showed parenchymal atrophy and replacement by lymphoid tissue, typical of the changes described by Cardell and Gurling.

CALCULUS FORMATION

These cases presented with a history of pain and swelling on the side of the calculus formation. There

FIG. 6. *Case 3. Biopsy of parotid gland (haematoxylin and eosin × 200) showing glandular atrophy with evidence of chronic inflammation.*

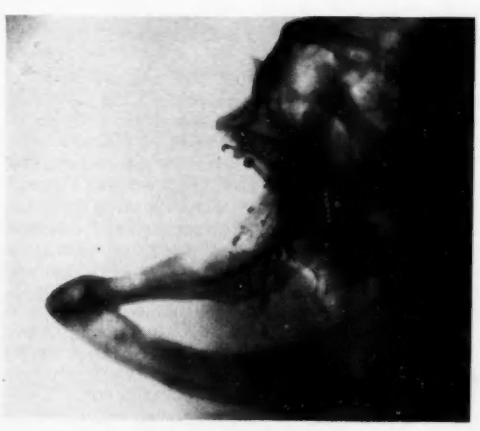

FIG. 7. *Sialogram of Case 4, showing scanty branch ducts with terminal dilatation.*

was evidence of infection in seven out of 14 cases but it was certainly not invariably present. For example, a man aged 43 noticed an acute, painful swelling of the left parotid gland while eating on two occasions, and on the second of these he pressed firmly over the swelling, which caused a sharp pain deep in the gland, followed by expulsion of a small phosphate calculus the size of a grape pip and a gush of pent-up secretion. Since this patient was seen, two other patients have been able to express stones by pressure in this way, one after nine years and the other after four years. Stones or grit were passed spontaneously in four other cases and in another a calculus was removed from the main duct at operation. Swellings were unilateral in all cases except two.

Sialography was carried out in 13 cases. In only two was a stone observed in a straight radiograph, but filling defects were present in the main duct in five and in branch ducts in two other patients. In two the main duct on the affected side was completely blocked. Dilatation of the main duct distal to the obstruction was demonstrated in eight cases, and localized dilatations of branch ducts in six (in

these both main duct and localized dilatations were present). Twelve patients were 37 years of age or more and the sex distribution was equal.

CASE 5 A man aged 37 complained of swelling of the left side of the face intermittently for one year. It was often made worse by eating and might persist for two weeks at a time. Pressure over the gland caused turbid fluid to exude from Stenson's duct. A calculus, approximately 1 cm. in length, could be felt at the edge of the masseter. A sialogram showed a convex filling defect in the main duct from which a radiotranslucent stone was subsequently removed (Fig. 8).

CASE 6 A man aged 59 had had recurrent swelling of the right parotid gland at irregular intervals during six or seven years. Small pieces of grit have been expelled from Stenson's duct from time to time. A sialogram showed a filling defect at the point of subdivision of the main duct with localized swelling of the branch duct (Fig. 9).

CASES WITH EVIDENCE OF ALLERGIC AETIOLOGY

These include 16 cases, 11 females and five males. Six patients, all women between the ages of 37 and 70 with bilateral swellings, had dilated main ducts, in one case limited to one side. The swellings appeared very frequently, sometimes during every meal. These cases have been described in detail elsewhere (Pearson, 1951). All gave a history of expelling mucus plugs followed by clear saliva from the duct orifices by pressure over the dilated gland, and microscopical examination showed that the plugs contained numerous eosinophils and in one polymorphs also. Four had asthma and two rhinorrhoea. All except one had a high eosinophil count in the blood. In some cases the eating of specific foods was considered by the patient to cause swelling, but this could not always be confirmed by observation. There is no reasonable doubt that eosinophilic mucus plugs which blocked the orifices of Stenson's ducts accounted for the swellings in these cases.

There were 10 other patients in whom allergy was thought to play some part in the aetiology. Three of these had terminal duct dilatations and five normal sialograms, including one of a patient with a history of many years' recurrent swellings. The ages of this group varied from 4 to 61 but only three were over 20. In none of these patients was purulent material ever seen emerging from Stenson's duct. The duration of episodes of swelling was brief in most cases. Four patients had asthma, two had had migraine at the same time as swellings were present, and one patient developed parotid swellings as part of a gold sensitivity reaction.

CASE 7 A girl aged 17 suffered from periodic swelling of both parotid glands for one day at a time. These swellings often followed the eating of egg in cake or pudding. Eggs have always caused vomiting, and eating a boiled egg was followed the next day by migraine, vomiting, and bilateral parotid swellings. A sialogram (Fig. 10) showed scattered dilatation of terminal ducts. She had no more swellings after the age of 19. The patient continues to avoid eggs.

SWELLINGS OF DOUBTFUL ORIGIN

This group includes 22 cases (11 males and 11 females). Twelve had bilateral swellings. Ten were under the age of 10, and six were over 30 years of

FIG. 8. *Case 5. Sialogram showing radiotranslucent stone in Stenson's duct.*

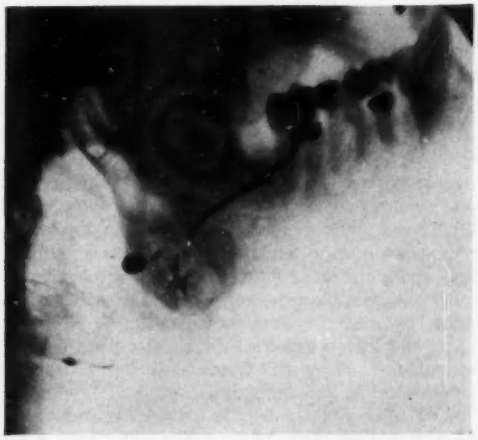

FIG. 9. *Case 6. Sialogram with filling defect due to calculus.*

FIG. 10. *Case 7 showing terminal duct dilatation in recurrent swelling of allergic origin.*

age. One woman of 66 had a dilated main duct, five had terminal dilatations, and eight had normal sialograms. Three were asthmatic. Two were siblings, and one other, a woman of 26, was the sister of a boy in the infected group. In some cases the swelling was almost certainly confined to the glandular or interstitial tissues and unassociated with duct obstruction.

TRAUMA TO DUCT ORIFICE

One patient, a woman of 58, was observed to have a lesion of the duct papilla on one side, probably caused by trauma from ill-fitting dentures.

DISCUSSION

Recurrent swellings of the parotid glands are not uncommon and present a difficult and complicated problem in pathogenesis. The tendency to develop bilateral swellings, often simultaneously, the changing sex ratio with increasing age accompanied by change in the sialographic appearances from dilated terminal ducts or acini in youth to dilatation of the main ducts in older people, all offer a tempting bait for speculation. There has been no lack of suggested aetiological factors, including infection (Payne, Rose, Jones), allergy (Pearson, 1935, 1936; Serafini, 1951; Waldbott and Shea, 1947; Findeisen, 1956; Riley, 1956), dental trauma (Rose, 1954), dehydration, diabetes (Lyon, 1943), improper mastication (Payne, 1940), heredity (Smith, 1953), auto-immune disease (Mosbech, Kristensen, 1960), and sialorrhoea (Reimann and Lindquist, 1952).

Parotid calculi are recognized but generally considered rare. In the present series of cases parotid calculi were identified in 14 patients. This was the only group of middle-aged or elderly patients in whom males were as numerous as females, and the only group in which unilateral swellings were more often seen than bilateral. The diagnosis is not always easy because the calculus may be small or radiotranslucent. Sialography may reveal a filling defect in the main or branch duct, with dilatation beyond it, and is an essential feature of the investigation of suspected cases. Infection may be present, but is probably secondary to obstruction in most cases.

In Sjögren's syndrome the dry mouth and poor salivary flow may be considered to account at least partly for the recurrent swellings found in this condition by predisposing to ascending infection. The reduction in size of the glands after treatment with corticotrophin suggests that there is also inflammatory swelling of the interstitial tissues (Gurling, Pearson, and Pond, 1954). The patients are almost exclusively female, and in the present series this was the only group of elderly women in whom dilatation of the terminal ducts was common. Main duct dilatation may also occur. The most characteristic feature of the sialogram is the small number of branch ducts. This is accounted for by atrophy of the gland and its replacement with fibro-adipose tissue (Cardell and Gurling, 1954).

Allergy can also reasonably be accepted as of some importance as an aetiological factor. The older patients are exclusively female and the swellings, which are chiefly of the main and branch ducts, are caused by eosinophilic plugs obstructing the orifices of Stenson's ducts from which they can readily be ejected by pressure. In these cases dilatation of the terminal ducts has not been seen. In the younger patients, however, males predominate and the sialographic appearances are either normal or with terminal duct dilatation. No patient under 30 was found to have a dilated main duct.

A similar relationship between age and sex and in the sialographic pattern is seen in the infected cases, and expression of mucous plugs containing polymorphs is sometimes encountered in the middle-aged women who mainly form this group. The organisms isolated from the purulent secretions in these patients are most commonly *Strep. viridans* and less frequently pneumococci and *Staph. aureus* (Payne, 1933, 1900). These findings have been confirmed in the present series.

It is difficult to understand why the pattern of duct obstruction should differ in patients of different ages,

even when the aetiological factors are not identical, *e.g.*, infection, allergy, and cases without obvious aetiology. There is general agreement that duct infection is almost invariably an ascending one. It seems that in young patients the infection may ascend rapidly into the branch ducts, where swelling of the mucosal lining of the intercalary ducts or of the interstitial tissue itself obstructs the lumen. Biopsy specimens from such cases confirm this view, revealing inflammatory change in the interstitial tissues with purulent material in small ducts. In these cases there is a definite tendency for improvement during adolescence when recurrent swellings disappear. Spontaneous remissions also take place in young patients with an allergic basis and those without obvious aetiological factors. In adults the main reaction to both ascending infection and allergic processes appears to be in the main duct, which responds by secretion of mucus and shedding of the lining cells of the duct to form masses which obstruct the main duct orifice causing it and its main branches to dilate. In other cases constrictions seen in the course of the main duct may indicate fibrous stricture. Biopsy of the gland in these cases shows little change in the acini and interstitial tissues. The tendency to form tenacious mucus is of course more apparent in the bronchial tract in older patients, especially those with bronchitis. It seems possible that the mucus-secreting glands which are found in Stenson's duct are also more active in this age group and pour out their secretions as a result of infective, allergic, or other stimuli.

Hereditary or congenital abnormalities of the ducts have been suggested as a possible basis for recurrent swellings. In the present series of cases there are two pairs of siblings, the first[1] a boy aged 12 with infective sialodochitis and terminal duct dilatation with early involvement of the main ducts, and his sister aged 26 years. She had had recurrent swellings as a child but at the time of investigation had been free of symptoms for some years but sialography still revealed bilateral terminal dilatation of the ducts.[1] The second pair were brothers aged 4½ and 1 year 9 months. Both were asthmatics but the aetiology of the swellings was considered uncertain and sialograms were not carried out. Two other patients who gave a history of unilateral swelling associated with infection had terminal duct dilatations on both sides. Rose quotes 10 cases in children between the age of 3 and 15 years who had advanced changes in the duct system in spite of a short and comparatively mild history, which he regards as evidence of pre-existing acinar dilatations. Smith (1953) records recurrent parotid swellings with radiological evidence of duct dilatation in three generations of a family.

Recurrent attacks of sialorrhoea may account for some of the transient bilateral swellings without evidence of infection or allergy (Reimann and Lindquist, 1952). Improper mastication with a consequently inadequate salivary flow (Payne, 1940) may well predispose to ascending infection, but this is difficult to determine. It may help to explain the onset of infection after dental extractions. Stenosis of the duct or duct papilla due to trauma from a faulty bite has been regarded as a relatively common cause of parotid swelling (Rose). Although the duct orifices are sometimes small and a cannula may be difficult to pass, especially when the orifice is on a mobile papilla, we have only encountered one case where there was evidence of trauma from a newly acquired set of dentures. The swelling was unilateral.

We have found no evidence that diabetes mellitus is a predisposing factor of importance. In spite of a careful search in the Diabetic Department at King's College Hospital, only one case of pneumococcal sialodochitis, in a diabetic boy aged 11, has been referred.

It is clear that there is a group of patients in whom the aetiological factor is obscure, and even when infection is present the underlying predisposing abnormality often remains unrecognized.

I should like to express my thanks to Dr. B. S. Cardell for help and advices.

[1]Previously reported (Pearson, 1935).

REFERENCES

Cardell, B. S., and Gurling, K. J. (1954). Observations on the pathology of Sjögren's syndrome. *J. Path. Bact.*, **68**, 137-146.

Findeisen, D. G. R. (1956). Allergy to dentures: A report of two cases presenting asthma and parotitis. *Int. Arch. Allergy*, **9**, 281-284.

Gurling, K. J., Pearson, R. S. B., and Pond, M. H. (1954). Sjögren's syndrome treated with A.C.T.H. *Brit. J. Opthal.*, **38**, 619-652.

Jones, H. Everley. (1953). Recurrent parotitis in children. *Arch. Dis. Childh.*, **28**, 182-186.

Lyon, E. (1943). Swelling of the parotid gland and diabetes mellitus. *Int. J. Gastroent.*, **68**, 139-147.

Mosbech, S., and Kristensen, H. P. O. (1960). Chronic Sialoadenitis caused by autoimmunisation. *Acta med. scand.*, **168**, 147-150.

Payne, R. T. (1933). Recurrent pyogenic parotitis. *Lancet*, **1**, 348-353.

—— (1940). Pneumococcal parotitis. *Brit. med. J.*, **1**, 287-292.

Pearson, R. S. B. (1935). Recurrent swelling of the parotid glands. *Arch. Dis. Childh.*, **10**, 363-376.

—— (1936). Two cases of recurrent swelling of the parotids. *Guy's Hosp. Rep.*, **86**, 333-342.

—— (1951). Parotid swelling due to allergic obstruction of the main duct. *Proceedings of First International Congress for Allergy, Basel*, pp. 868-873.

Riley, H. D. (1956). Recurrent parotid swelling. *Sth. med. J. (Bgham, Ala.)*, **49**, 523-528.

Rose, S. S. (1954). A clinical and radiological survey of 192 cases of recurrent swellings of the salivary glands. *Ann. roy. Coll. Surg.*, **15**, 374-401.

Reimann, H. A., and Lindquist, J. N. (1952). Periodic sialorrhea. *J. Amer. med. Ass.*, **149**, 1465-1467.

Serafini, U. (1951). Prime osservazioni su alcuni casi di tumefazione acuta ricorrente della parotide di probabile origine allergica. *Minerva med. (Torino)*, **17**, 271-275.

Smith, M. (1953). Familial incidence of sialectatis. *Brit. med. J.*, **2**, 1359.

Waldbott, G. L., and Shea, J. J. (1947). Allergic parotitis. *J. Allergy*, **18**, 51-54.

Gut, 1961, **2**, 218

$Co^{58}B_{12}$ absorption (hepatic surface count) after gastrectomy, ileal resection, and in coeliac disorders

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SYNOPSIS This paper demonstrates that the hepatic surface counting technique is a reliable and satisfactory method of estimating the absorption of radioactive vitamin B_{12}. It does not require the collection of faeces or urine and is therefore particularly useful in studying out-patients.

When $Co^{58}B_{12}$ is absorbed from the intestinal tract, radioactivity accumulates in the liver, reaching its maximum in seven to 10 days and thereafter slowly declines. Glass, Boyd, Gellin, and Stephanson (1954) demonstrated that the degree of absorption of vitamin B_{12} could be measured by scintillation counting over the surface of the liver and that hepatic surface radioactivity was a true measure of the content of radioactive B_{12} in the liver. Baker and Mollin (1956) demonstrated good correlation between the hepatic uptake measured by direct counting over the liver and the amount of radioactive B_{12} absorbed as estimated by the faecal excretion technique.

The method has certain practical advantages compared with the more widely used faecal (Heinle, Welch, Scharf, Meacham, and Prusoff, 1952) and urinary excretion methods (Schilling, 1953). It does not require the collection of faeces or urine and should therefore be of particular value for the investigation of out-patients, and secondly it does not require the administration of a large saturating dose of non-radioactive vitamin B_{12} which may obscure the haematological picture and response. Despite these advantages it does not appear to have been adopted generally in Britain. The purpose of this paper is to check the validity of the technique and to assess its use in three common groups of intestinal disorders in which abnormalities of B_{12} absorption may occur, namely, post-gastrectomy states, intestinal resection, and the coeliac group of disorders.

METHODS

Co^{58}-labelled vitamin B_{12} (supplied by the Radiochemical Centre, Amersham, England) was administered orally in a dose of 0·25 to 0·50 μg. of vitamin B_{12} containing 0·33-0·50 μc. Co^{58}, dissolved in 20 ml. distilled water after patients had fasted for at least 12 hours (overnight); no food or drink was allowed for the following two hours.

The technique of estimating the accumulated hepatic radioactivity was essentially similar to that described by Glass and his co-workers. The hepatic radioactivity was counted with an EKCO cylindrically collimated scintillation counter having a $1\frac{1}{2}$ in. thallium-activated sodium iodide crystal. Initially, counting was performed in two or more planes over the liver surface, but it was found that the variation in counts obtained in different planes was small, and that entirely satisfactory results were obtained by counting in one only. The majority of tests were, therefore, performed with the collimator placed in contact with the skin in the anterior axillary line in the centre of the area of liver dullness. Counting was carried out for five minutes, the results obtained then being corrected for background radiation and expressed as counts per minute per 1 μc. of Co^{58}.

The liver was counted in this manner before administration of the dose, and then again between the sixth and tenth days afterwards. Initially, two counts were made on different days in this period to ensure that the plateau had been reached; it soon became evident that a single count was quite satisfactory, and subsequently this was performed, usually on the seventh day.

When the absorption of $Co^{58}B_{12}$ was found to be impaired, a second dose was given with 10 mg. of intrinsic factor concentrate (Lederle) active in pernicious anaemia in 5 to 10 mg. quantities. The hepatic surface count was then repeated seven days later. The difference between the two readings (after background corrections) was taken to represent the hepatic radioactivity resulting from increased absorption of vitamin B_{12} as a result of the addition of intrinsic factor. On each occasion the radioactivity over the lower abdomen (just below the umbilicus) was also measured. If the counts in this region exceeded 50% of the liver count, the estimation was repeated after an interval of at least three days. This was, however, only occasionally necessary.

Serum levels of vitamin B_{12} were estimated by the method of Meynell, Cooke, Cox, and Gaddie (1957), which gives a normal range of 105 to 672 $\mu\mu$g. per ml. with a mean normal concentration of 281 $\mu\mu$g. per ml.

The faecal excretion of the administered $Co^{58}B_{12}$ was estimated by counting the radioactivity appearing in the faeces daily until none was present on each of two consecutive days. The total faecal radioactivity was expressed as a percentage of the dose (after background corrections) obtained in the same counter.

RESULTS

CORRELATION OF HEPATIC SURFACE COUNT WITH FAECAL EXCRETION OF $Co^{58}B_{12}$

On 15 occasions the percentage absorption of the administered dose of $Co^{58}B_{12}$ was estimated by the faecal excretion method at the same time as the hepatic radioactivity was measured (Fig. 1). The results indicate good correlation between these two methods.

CONTROL GROUPS

NORMAL SUBJECTS (FIG. 2) This group of 28 subjects consisted of 20 hospital patients with no gastrointestinal or haematological disorder, five with iron-deficiency anaemia, and three with ulcerative colitis but with no evidence of small intestinal involvement. All had normal serum levels of vitamin B_{12}, and no clinical evidence of B_{12} deficiency; hepatic radioactivity ranged from 1,600 to 4,633 counts per minute per microcurie with a mean of 2,914, S.D. 828; statistical range (mean $\pm$ 2 S.D.) 1,258-4,570. The test was repeated with the addition of intrinsic factor in six subjects. In each case the result was similar to that obtained by the ingestion of $Co^{58}B_{12}$ alone.

PERNICIOUS ANAEMIA (FIG. 2) Nineteen patients with Addisonian anaemia were studied. In 12 the diagnosis had previously been established on adequate grounds and confirmed by continued normal haematological values with liver or vitamin B_{12} therapy over periods of up to 10 years. In the remaining seven the test was performed as part of the initial diagnostic investigation. None of these patients had steatorrhoea. Following ingestion of $Co^{58}B_{12}$ alone, hepatic surface counts varied from 0 to 720 per minute per microcurie (mean 237, S.D. 231). When intrinsic factor was added to the ingested $Co^{58}B_{12}$, the hepatic surface counts were increased in each

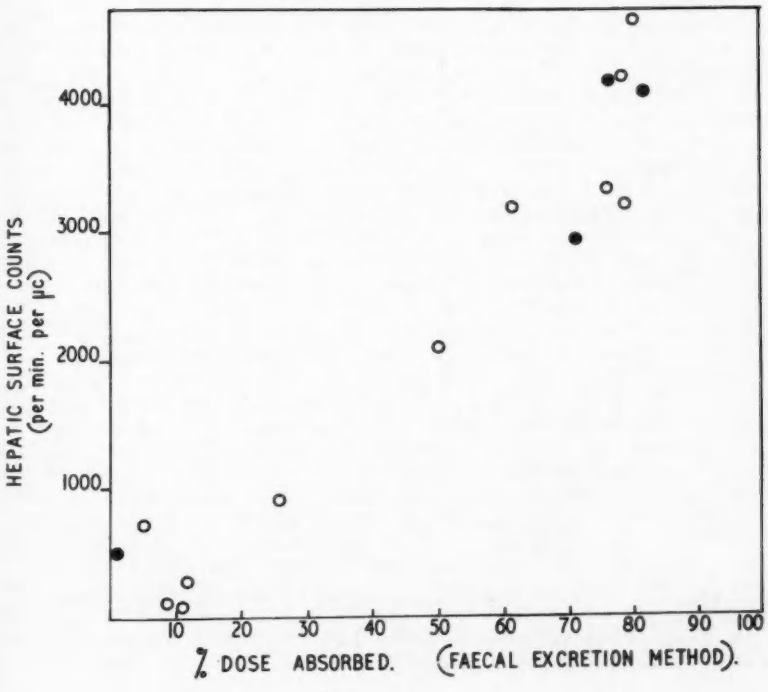

FIG. 1. *Hepatic surface radioactivity compared with the percentage of $Co^{58}B_{12}$ absorbed as determined by the faecal excretion technique.*
Open circles represent a test performed with Co^{58} vitamin B_{12} alone.
Closed circles represent a test performed with Co^{58} vitamin B_{12} plus intrinsic factor.

case to the same range as obtained in the normal subjects (range 1,574 to 4,268).

TOTAL GASTRECTOMY Two patients were studied four and 10 years after total gastrectomy. Both had received regular B_{12} therapy since the time of operation. In both the hepatic surface counts following ingestion of $Co^{58}B_{12}$ alone were very low (0 and 66), but on the addition of intrinsic factor increased to the normal range (2,365 and 2,172).

INTESTINAL DISORDERS

PARTIAL GASTRECTOMY Twenty-four patients were studied following partial gastrectomy, the interval since operation being three months to 12 years (Fig. 2). In 18 the results were within the normal range. None of these patients had any clinical manifestation of B_{12} deficiency, and in the majority serum vitamin B_{12} levels have been persistently normal; occasional moderately low levels of serum vitamin B_{12} have been encountered in three of these subjects in whom absorption was found to be normal.

In four cases the hepatic surface counts were in the same range as seen in pernicious anaemia (261, 282, 292, 723), and in each increased to the normal range upon the addition of intrinsic factor to the ingested $Co^{58}B_{12}$ (2,799, 3,000, 1,907, 2,919). These four patients had had a Polya type gastrectomy seven, nine, 10, and 12 years previously, in two instances for gastric carcinoma and in two for chronic peptic ulcer. They have all developed clinical evidence of deficiency of vitamin B_{12}. Three developed megaloblastic anaemia (seven, nine, and 12 years after operation), associated with very low serum B_{12} levels and responded adequately to B_{12} therapy. The fourth developed subacute combined degeneration of the spinal cord 10 years after operation, associated with a very low serum B_{12} level. He, also, responded excellently to B_{12} therapy.

In two patients the hepatic surface counts were below normal (1,028 and 1,034 counts per minute

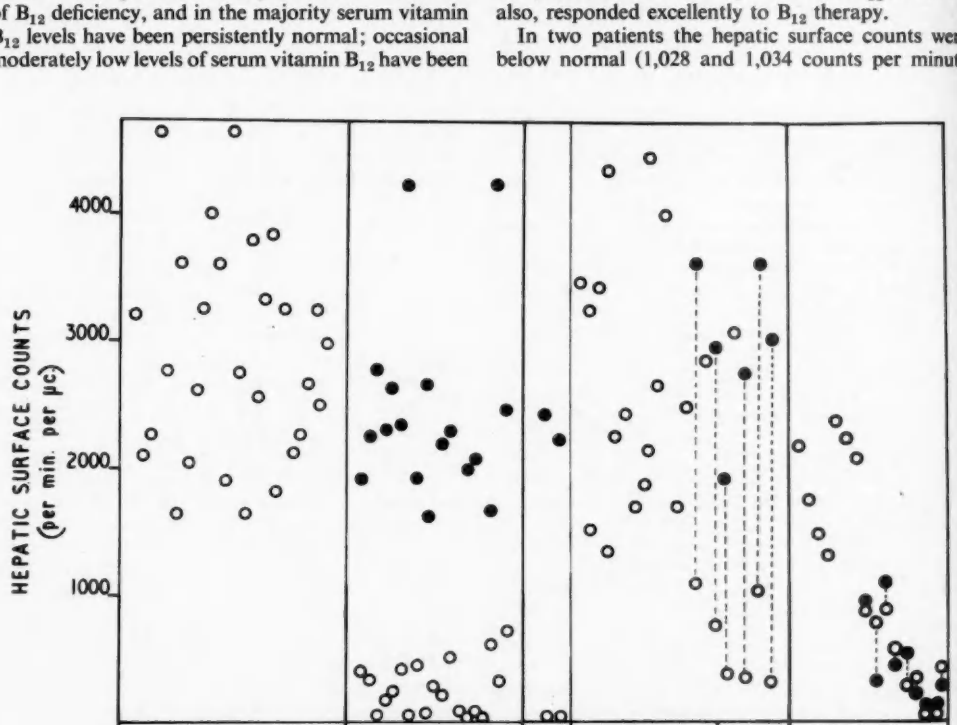

FIG. 2. *Hepatic surface radioactivity in control subjects and in patients with pernicious anaemia, following gastrectomy and resection of the ileum.*
Open circles represent a test performed with Co^{58} vitamin B_{12} alone.
Closed circles represent a test performed with Co^{58} vitamin B_{12} plus intrinsic factor.

per microcurie) but not as low as those seen in pernicious anaemia. In each the count rose considerably after the addition of intrinsic factor to the ingested dose (3,600 and 3,646 counts per minute per microcurie). In one, megaloblastic anaemia occurred five years after partial gastrectomy for gastric and duodenal ulcers; full response followed vitamin B_{12} therapy, which was discontinued after three years. Two years later macrocytosis and glossitis recurred, which again responded to vitamin B_{12} therapy. The other patient was seen eight years after operation with a severe iron-deficiency anaemia and an associated low serum B_{12} level of 50 $\mu\mu$g. per ml. Her marrow showed normoblastic erythropoiesis with no evidence of megaloblastic change.

AFTER RESECTION OF THE ILEUM Sixteen patients were studied after resection of a portion of the ileum (Fig. 2). In 14 this resection was performed for regional ileitis (right hemicolectomy in 11, resection of the distal ileum alone in two, and resection of a segment of the mid-ileum in one). In one patient a right hemicolectomy was performed for carcinoma of the caecum; in one 24 in. of distal ileum was resected for an argentaffin tumour.

In seven patients, in whom resection was performed from one to 14 years previously, the hepatic surface radioactivity was within the normal range. All seven have maintained normal serum vitamin B_{12} levels and none has developed macrocytic anaemia.

In the other nine patients in whom resection was performed from one to 13 years previously, the hepatic radioactivity was below normal, varying from 60 to 876 counts per minute per microcurie after the ingestion of $Co^{58}B_{12}$. When intrinsic factor was added to the $Co^{58}B_{12}$, the counts were not significantly altered (72-1,156). In two cases a third dose was given following the administration of tetracycline for five days: in neither was there any significant improvement in the absorption. These nine patients have all had abnormally low serum vitamin B_{12} levels since their operation (25 to 85 $\mu\mu$g. per ml.). Six were receiving parenteral vitamin B_{12} therapy; in three this was begun because of the development of macrocytic anaemia (the marrow was megaloblastic in the two patients in whom it was examined), and in three because of persistently low serum vitamin B_{12} concentrations, though anaemia was not present. Three have not yet received vitamin B_{12} therapy, though they also have persistently low serum B_{12} levels but no anaemia.

The area of ileum resected in the patients who have retained normal vitamin B_{12} absorption was the terminal ileum in six, the lengths being 6 in., 9 in., 12 in., 12 in., and 4 ft. (it was unknown in one case); the seventh patient had 16 in. of mid-ileum removed.

The nine patients with impaired vitamin B_{12} absorption all had terminal ileum resected, the lengths in seven being 12 in., 18 in., 2 ft., 2 ft., 3 ft., 6 ft., and 'a few feet'. In one 11 in. of terminal ileum was resected as well as a further 9 in. about 2 ft. proximally. The ninth had a right hemicolectomy for carcinoma of the caecum.

None of the 14 patients with Crohn's disease has had clinical evidence of recurrence, and in none is there evidence of stricture or formation of a blind loop. Only two have biochemical evidence of active disease with increased serum seromucoid concentration (Cooke, Fowler, Cox, Gaddie, and Meynell, 1958). In one of these absorption is normal, in the other it is impaired. Liver function tests were normal in all.

ADULT COELIAC DISEASE AND IDIOPATHIC STEATORRHOEA Fifty patients were studied in whom a diagnosis of adult coeliac disease (Cooke, 1958) or 'idiopathic steatorrhoea' (Cooke, Peeney, and Hawkins, 1953) had been made. The results of the hepatic radioactivity are shown in Fig. 3, in which these patients are classified according to criteria recently described (Fone, Cooke, Meynell, Brewer, Harris, and Cox, 1960) with special reference to the histological appearances of the upper small intestine obtained by peroral biopsy.

In 22 patients with adult coeliac disease and a 'flat' jejunal mucosa, the hepatic radioactivity was within the normal range in 18; in four it was below normal (in three as low as that seen in pernicious anaemia), but was not significantly improved by the addition of intrinsic factor.

On the 28 patients with jejunal biopsies showing 'abnormal villi' (partial atrophy), eight resembled those of adult coeliac disease in many respects but differed particularly in the histology of the jejunal mucosa. Hepatic radioactivity was normal in five; in three it was below normal, but improved to the normal range when intrinsic factor was added. These three each had histamine-fast achlorhydria, and in the two in whom gastric biopsy was performed the mucosa showed severe inflammation and glandular atrophy.

In seven patients the major feature was a severe megaloblastic anaemia associated with low serum levels of vitamin B_{12} which responded adequately to parenteral B_{12} therapy. In five hepatic radioactivity was much below normal, but was improved to the normal range by the addition of intrinsic factor. All five had histamine-fast achlorhydria, and severe gastric atrophy was present in all four in whom biopsy was performed. One patient had normal hepatic radioactivity; his gastric mucosa was normal. The seventh patient had impaired absorption which

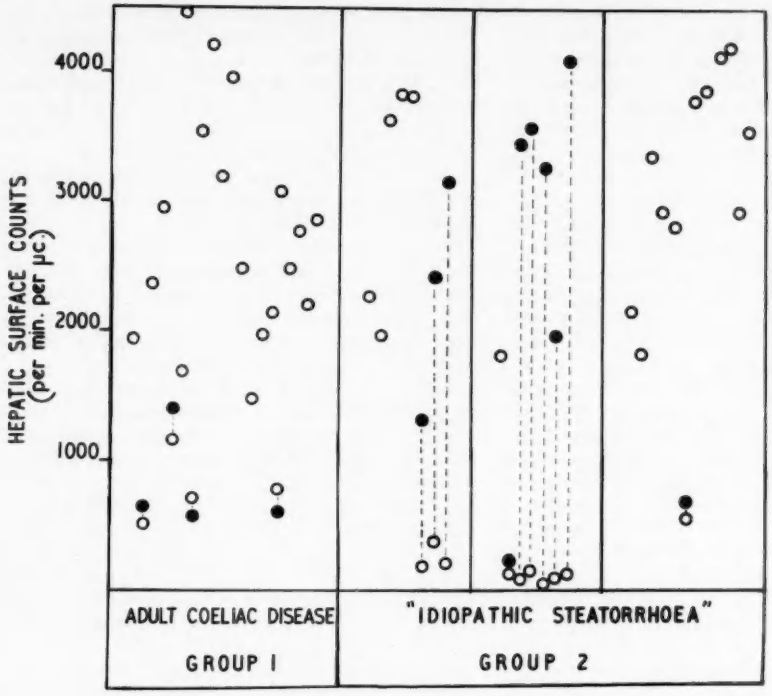

FIG. 3. *Hepatic surface radioactivity in patients with 'idiopathic steatorrhoea'.*
Open circles represent a test performed with Co^{58} *vitamin* B_{12} *alone.*
Closed circles represent a test performed with Co^{58} *vitamin* B_{12} *plus intrinsic factor.*

was not improved by the addition of intrinsic factor.

In 12 patients the major clinical feature was a severe megaloblastic anaemia associated with normal serum vitamin B_{12} levels, free gastric HCl; it responded adequately to folic acid therapy. In 11 the hepatic radioactivity was normal. The gastric mucosa was normal in the three examined. In one patient hepatic radioactivity was low and not improved by intrinsic factor; he also had a normal gastric mucosa.

DISCUSSION

An intrinsic factor defect was clearly demonstrated in all our patients with pernicious anaemia, whilst the complete lack of intrinsic factor secretion expected to follow total gastrectomy was confirmed in our two patients. As was shown by Baker and Mollin (1956), there is good correlation with the faecal excretion method of Heinle *et al.* (1952), hence the hepatic radioactivity after an oral dose of radjoactive vitamin B_{12} appears to be closely related to the amount absorbed. Our results in the control groups confirm previous observations that the hepatic surface counting technique is a reliable method of estimating the degree of absorption of radioactive vitamin B_{12}.

A varying degree of impairment of intrinsic factor secretion is a recognized, but uncommon, sequel of partial gastrectomy (Badenoch, Evans, Richards, and Witts, 1955; MacLean, 1957; Loewenstein, 1958) and was seen in six of our 24 patients. Each of these six had developed evidence of B_{12} deficiency. As these cases did not form an unselected series, the results do not reflect the true incidence of B_{12} deficiency as a result of partial gastrectomy which has been estimated as 0·6% (MacLean, 1957).

Since partial gastrectomy does not remove much of the intrinsic factor secreting area of the mucosa—fundus and body—as does total gastrectomy, some other factor must be involved. Badenoch *et al.* (1955), on the basis of gastric biopsy studies in five patients, suggested that persistence of gastritis with mucosal atrophy in the gastric remnant is the explanation of the intrinsic factor defect. A similar conclusion was reached by MacLean (1957) in a study of the mucosa of resected stomachs; he suggested that the development of megaloblastic anaemia following partial gastrectomy might be predicted by such study.

The observation of moderately low serum B_{12}

levels despite normal B_{12} absorption in three patients following partial gastrectomy is of interest. As reported by Cox *et al.* (1959), the serum levels in such patients do return to normal with the administration of iron. A possible explanation might be that although the B_{12} is absorbed when given under the 'ideal' conditions of the test, it may not be absorbed as well when presented in food. This phenomenon has been demonstrated with the absorption of iron (Baird and Wilson, 1959), and may apply to B_{12} as well. Glass *et al.* (1960) have found low serum B_{12} levels in some cases of gastritis with histamine-fast anacidity in which absorption of radioactive B_{12} was normal. They conclude that the explanation for these inter-relationships must await more complete knowledge of the natural history of gastric atrophy and B_{12} deficiency.

Impaired absorption which was not corrected by the addition of intrinsic factor was demonstrated in nine of the 16 cases following resection of ileum. In all 16 cases the degree of absorption of vitamin B_{12} was in excellent agreement with other features of vitamin B_{12} metabolism. All seven patients in whom normal absorption was demonstrated had maintained normal serum B_{12} levels and normal blood pictures since operation: the nine with impaired absorption had all developed low serum vitamin B_{12} levels with macrocytic anaemia in three. It is generally believed that in man vitamin B_{12} is absorbed in the distal small intestine. Booth and Mollin (1959) found that absorption of vitamin B_{12}, with and without intrinsic factor, was invariably subnormal in the patients who had had more than 6 to 8 ft. of ileum resected or short circuited; the terminal ileum was involved in each of their cases. In general, the length of resected ileum was greater in our patients with impaired absorption than in those in whom absorption remained normal. It is of particular interest, however, that absorption was impaired in two patients in whom only 12 in. and 18 in. of terminal ileum was resected. All but two of our group had had the resection performed for Crohn's disease; there was no significant difference between those with normal and those with abnormal absorption as regards severity of the lesion before operation or incidence of recurrence after operation. The diminution in absorption appears more closely related to the extent of involvement by the disease process than from intestinal stasis or blind loops. These cases provide further evidence that the distal ileum is the most important site for absorption of vitamin B_{12} in man. They also emphasize the need for constant observation for the occurrence of vitamin B_{12} deficiency or alternatively the routine prophylactic administration of vitamin B_{12} after resection of even short lengths of ileum.

A similar type of absorptive defect was found in only six of 50 patients with adult coeliac disease and 'idiopathic steatorrhoea'. Contrary to the findings of Oxenhorn, Estren, Wasserman, and Adlersberg (1958) and Glass (1959), absorption of radioactive B_{12} was found to be normal in the majority of our cases of adult coeliac disease, and these observations correlate well with the maintenance of normal serum B_{12} levels in the majority and the absence in this group of any instance of megaloblastic anaemia due to vitamin B_{12} deficiency.

A total of 11 patients with 'idiopathic steatorrhoea', *i.e.*, with abnormal jejunal villi or 'partial atrophy', had a megaloblastic anaemia due to vitamin B_{12} deficiency; in all but one the deficiency was due to impaired secretion of intrinsic factor. The combination of intrinsic factor defect and steatorrhoea has been previously described by Mollin, Booth, and Baker (1957), Järvinen and Latvalahti (1958), Williams, Coghill, and Edwards (1958), and Doig and Girdwood (1960). We consider these patients to be a group distinct from adult coeliac disease. They also differ from patients with pernicious anaemia, whom they resemble clinically in that dysfunction of the small intestine can be demonstrated (Fone, Cooke, Meynell, and Cox, 1961).

One patient, a man of 39 years, was seen with megaloblastic anaemia, low serum vitamin B_{12}, and normal gastric mucosa in whom B_{12} absorption was found on two occasions to be normal. Haematological response to B_{12} therapy was complete. There was no history of anticonvulsant therapy. We have no adequate explanation for this finding at present, and he is the only patient in this series in whom the hepatic radioactivity could be considered inconsistent with other features of vitamin B_{12} metabolism.

SUMMARY AND CONCLUSIONS

The hepatic surface counting technique is a reliable and satisfactory method of estimating the absorption of radioactive vitamin B_{12}. It does not require collection of faeces or urine, or the administration of a flushing dose of non-radioactive vitamin B_{12}. It is therefore particularly useful for the study of outpatients, and its convenience and accuracy justify more general use.

Results in patients following partial gastrectomy were comparable to those reported by others. Nine of 16 patients who had undergone ileal resection mainly for regional ileitis had impaired absorption of $Co^{58}B_{12}$, not improved with the addition of intrinsic factor. There was some general relationship between the degree of impairment and the amount of intestine resected. In contradistinction to other reports, only

six out of 50 patients with adult coeliac disease and 'idiopathic steatorrhoea' showed this type of impaired absorption of $Co^{58}B_{12}$ but 10 with abnormal villi (partial atrophy) demonstrated an intrinsic factor deficit as the cause for their failure to absorb $Co^{58}B_{12}$.

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REFERENCES

Badenoch, J., Evans, J. R., Richards, W. C. D., and Witts, L. J. (1955). Megaloblastic anaemia following partial gastrectomy and gastroenterostomy. *Brit. J. Haemat.*, **1**, 339-344.

Baird, I. M., and Wilson, G. M. (1959). The pathogenesis of anaemia after partial gastrectomy. II. Iron absorption after partial gastrectomy. *Quart. J. Med.*, n.s. **28**, 35-41.

Baker, S. J., and Mollin, D. L. (1956). The relationship between intrinsic factor and the intestinal absorption of Vitamin B_{12}. *Brit. J. Haemat.*, **1**, 46-51.

Booth, C. C., and Mollin, D. L. (1959). The site of absorption of Vitamin B_{12} in man. *Lancet*, **1**, 18-21.

Cooke, W. T. (1958). Adult coeliac disease and other disorders associated with steatorrhoea. *Brit. med. J.*, **2**, 261-265.

——, Fowler, D. I., Cox, E. V., Gaddie, R., and Meynell, M. J. (1958). The clinical significance of seromucoids in regional ileitis and ulcerative colitis. *Gastroeneterology*, **34**, 910-919.

——, Peeney, A. L. P., and Hawkins, C. F. (1953). Symptoms, signs and diagnostic features of iodbpathic steatorrhoea. *Quart. J. Med.*, **22**, 59-77.

Cox, E. V., Meynell, M. J., Gaddie, R., and Cooke, W. T. (1959). Inter-relation of vitamin B_{12} and iron. *Lancet*, **2**, 998.

Doig, A., and Girdwood, R. H. (1960). The absorption of folic acid and labelled cyanocobalamin in intestinal malabsorption. *Quart. J. Med.*, n.s. **29**, 333-374.

Fone, D. J., Cooke, W. T., Meynell, M. J., Brewer, D. B., Harris, E. L., and Cox, E. V. (1960). Jejunal biopsy in adult coeliac disease and allied disorders. *Lancet*, **1**, 933-939.

——, ——, ——, and Cox, E. V. (1961). To be published.

Glass, G. B. Jerzy (1959). Deposition and storage of vitamin B_{12} in the normal and diseased liver. *Gastroenterology*, **36**, 180-192.

——, Boyd, L. J., Gellin, G. A., and Stephanson, L. (1954). Uptake of radioactive vitamin B_{12} by the liver in humans: test for measurement of intestinal absorption of vitamin B_{12} and intrinsic factor activity. *Arch. Biochem. Biophys.*, **51**, 251-257.

——, Speer, F. D., Nieburgs, H. E., Ishimori, A., Jones, E. L., Baker, H., Schwartz, A., and Smith, R. (1960). Gastric atrophy, atrophic gastritis, and gastric secretory failure. *Gastroenterology*, **39**, 429.

Heinle, R. W., Welch, A. D., Scharf, V., Meacham, G. C., and Prusoff, W. H. (1952). Studies of excretion (and absorption of Co^{60} labelled vitamin B_{12} in pernicious anaemia). *Trans. Ass. Amer. Phycns*, **65**, 214-222.

Järvinen, K. A. J., and Latvalahti, J. (1958). Some observations on non-tropical sprue: A case with hypocalcemia, intrinsic factor deficiency, anaemia and diabetes mellitus. *Ann. Med. intern. Fenn.*, **47**, 39-46.

Loewenstein, F. (1958). Absorption of cobalt^{60} labelled vitamin B_{12} after sub-total gastrectomy. *Blood*, **13**, 339-347.

MacLean, L. D. (1957). Incidence of megaloblastic anaemia after sub-total gastrectomy. *New Engl. J. Med.*, **257**, 262-265.

Meynell, M. J., Cooke, W. T., Cox, E. V., and Gaddie, R. (1957). Serum cyanocobalamin level in chronic intestinal disorders. *Lancet*, **1**, 901-904.

Mollin, D. L., Booth, C. C., and Baker, S. J. (1957). The absorption of vitamin B_{12} in control subjects, in Addisonian pernicious anaemia and in the malabsorption syndrome. *Brit. J. Haemat.*, **3**, 412-428.

Oxenhorn, S., Estren, S., Wasserman, L. R., and Adlersberg, D. (1958). Malabsorption syndrome: intestinal absorption of vitamin B_{12}. *Ann. intern. Med.*, **48**, 30-38.

Schilling, R. F. (1953). Intrinsic factor studies. The effect of gastric juice on the urinary excretion of radioactivity after the oral administration of radioactive vitamin B_{12}. *J. Lab. clin. Med.*, **42**, 860-866.

Williams, A. W., Coghill, N. F., and Edwards, F. (1958). The gastric mucosa in pernicious anaemia: biopsy studies. *Brit. J. Haemat.*, **4**, 457-464.

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Comparative investigations on the activity of leucine aminopeptidase, glutamic oxalacetic transaminase, and alkaline phosphatase in serum

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SYNOPSIS These studies on leucine aminopeptidase demonstrate that it has the same significance as alkaline phosphatase in relation to jaundice.

During recent years the leucine aminopeptidase activity in serum has been the object of increasing interest (Fleisher and Butt, 1953; Green, Kwan-Chung Tsou, Bressler, and Seligman, 1955; Fleisher, Butt, and Huizenga, 1957; Goldbarg and Rutenburg, 1958; Rutenburg, Goldbarg, and Pineda, 1958; Braun-Falco and Salfeld, 1957; Arst, Manning, and Delp, 1959; Goldbarg, Pineda, and Rutenburg, 1959; Kowlessar, Haeffner, and Sleisenger, 1960). The clinical significance of raised levels of activity is still a matter of dispute, however, and systematic, comparative investigations of the correlation of leucine aminopeptidase activity with alkaline phosphatase activity and glutamic oxalacetic transaminase activity are not available.

The object of the present work is to contribute towards the elucidation of the clinical significance of raised serum leucine aminopeptidase activity and to compare its diagnostic value with that of the alkaline phosphatase and the glutamic oxalacetic transaminase activity in serum. For this purpose simultaneous determinations of the serum activities of the three enzymes mentioned have been made in a number of patients.

Leucine aminopeptidase is an exopeptidase belonging to the aminopeptidases (Smith, 1951). Its specificity is low (Smith and Spackman, 1955; Hill and Smith, 1957), and it has been demonstrated in most mammalian tissues (Goldbarg and Rutenburg, 1958; Rutenburg *et al.*, 1958; Willighagen and Planteydt, 1959; Burstone and Folk, 1956; Adams, McFadden, and Smith, 1952; Pearse and Tremblay, 1958).

[1]Aided by a grant from the Danish Cancer Society.

RESULTS OF PREVIOUS CLINICAL INVESTIGATIONS

In an investigation of the peptidase content of human serum Fleisher and Butt (1953) found raised leucine aminopeptidase activity in hepatobiliary diseases. In a large group of patients, Fleisher *et al.* (1957) found the greatest increase in activity in acute epidemic hepatitis and less in neoplastic liver disease, bile duct obstruction, and portal cirrhosis. Goldbarg and Rutenburg (1958) and Rutenburg *et al.* (1958) found the highest leucine aminopeptidase activity in serum from patients with carcinoma of the head of the pancreas and significantly lower activity in choledocholithiasis and liver metastases. Arst *et al.* (1959) found, as did others (Braun-Falco and Salfeld, 1957), the highest activity in pregnant women during the last trimester. These authors also found considerably increased serum activities in hepatobiliary diseases, but they are not of the opinion that the analysis provides any differential diagnostic information within this disease group. Braun-Falco and Salfeld (1957) found increased leucine aminopeptidase activity in pemphigus vulgaris and in acute eczematous diseases.

METHODS

The leucine aminopeptidase activity in serum and bile was measured according to the method devised by Goldbarg and Rutenburg (1958).

The alkaline phosphatase activity in serum was determined according to the method devised by Kind and King (1954) and is stated in King-Armstrong units per 100 ml. serum.

The glutamic oxalacetic transaminase activity in serum was determined according to the method devised by

Henley and Pollard (1955) and is stated in units per ml. serum.

Where the problem of significance is mentioned below, $p = 0.05$ is taken to be the limiting value.

CLINICAL MATERIAL

Normal values for leucine aminopeptidase activity as well as for glutamic oxalacetic transaminase and alkaline phosphatase activity in serum were determined on the basis of analytical results from a group of patients selected in such a manner that those with clinical, biochemical, or signs at necropsy of hepatobiliary disease or congestive heart failure were excluded.

CHOLEDOCHOLITHIASIS This group comprises 16 patients with 17 admissions to the department. In 10 cases the diagnosis was confirmed by operation and in the remaining cases it is based on clinical and radiological examinations.

CHOLECYSTOLITHIASIS This group comprises 14 patients in whom the diagnosis was confirmed by operation or *x*-ray examination. Six out of the 14 were described as complicated by cholecystitis or cholangitis.

CARCINOMA OF THE PANCREAS This occurred in 11 cases in all. In eight patients the disease was found in the head of the pancreas, and four of them had metastases in the liver. In another three patients, the disease was found in the body of the pancreas accompanied by liver metastases in one, and in two in the tail of the pancreas, one of these with liver metastases.

LIVER METASTASES These were observed in 12 patients, the diagnosis being in all cases confirmed at necropsy. Patients with the pancreas as the site of the primary tumour are not included in this group.

CHRONIC HEPATITIS This group comprises 20 patients with 22 admissions to the department. In 12 cases the diagnosis was confirmed by liver biopsy, while in the remaining eight it was based solely on clinical and biochemical findings.

ACUTE HEPATITIS This is a group of six patients with typical clinical findings.

MYOCARDIAL INFARCTION This group comprises 37 patients with typical clinical findings and pathognomonic alterations in the electrocardiographic readings in the course of the disease. Seventeen of these patients died, necropsy was performed in 16, and the diagnosis was confirmed in all. The mean values of the three enzymatic activities within this group were calculated on the basis of all the analyses in which a raised glutamic oxalacetic transaminase activity was observed, as well as on the basis of the analyses corresponding to maximum transaminase activity in the patients in whom all transaminase analyses gave results within the normal range. In nine of the 37 patients an enlarged liver due to congestive heart failure was observed, in six cases at necropsy and in three by palpation. These patients are classified separately.

RESULTS

GLUTAMIC OXALACETIC TRANSAMINASE The highest activity was found in patients with acute hepatitis (epidemic). In intra- and extrahepatic biliary obstruction and in myocardial infarction a moderate increase in the glutamic oxalacetic transaminase activity was observed. In patients with liver metastases normal activity was found in five out of 12 cases, but, when 10 cases of carcinoma of the pancreas with liver metastases are included, the frequency of normal activity was only five out of 22 cases.

ALKALINE PHOSPHATASE In this study the highest alkaline phosphatase activity was observed in cases of choledocholithiasis, carcinoma of the head of the pancreas (extrahepatic biliary obstruction), and in patients with liver metastases (intrahepatic biliary obstruction). There is no significant difference between the mean values obtained in these clinical groups.

Moderately raised alkaline phosphatase activity was found in serum from patients with acute (epidemic) hepatitis, chronic hepatitis, and cholecystolithiasis complicated by cholecystitis and cholangitis. The mean values for these diseases do not show any significant difference, but they are all significantly lower than the mean values prevailing in the case of intra- and extrahepatic biliary obstruction.

In myocardial infarction and in uncomplicated cholecystolithiasis the serum alkaline phosphatase activity was found to be normal.

Comparisons between the analytical results obtained for the alkaline phosphatase activity, glutamic oxalacetic transaminase activity, and bilirubin concentration in serum from patients with extra- or intrahepatic biliary obstruction (40 patients) show that all of the 62 alkaline phosphatase analyses gave pathological values, that 15 out of 71 glutamic oxalacetic transaminase analyses gave normal results ($\leq$ 2·0 units/ml.), and that 11 out of 44 bilirubin concentrations were $\leq$1·0 mg.% (Table I).

TABLE I

DISSOCIATION BETWEEN ANALYTICAL VALUES ON SERUM FROM 40 PATIENTS WITH INTRA- OR EXTRAHEPATIC BILIARY OBSTRUCTION

	Full Analysis	*Normal*	*Pathological*
Alkaline phosphatase	62	0	62
Leucine aminopeptidase	71	0	71
Go-T.	71	15	56
Serum bilirubin	44	11	33

The 62 alkaline phosphatase analyses from patients with biliary obstruction and the 36 analyses from patients with parenchymatous liver diseases (acute and chronic hepatitis) are distributed about the value of 30 K.-A. units/100 ml. serum in such a manner that 16% of the values obtained in cases of obstructive disease are below this value and 14% of the values obtained in cases of parenchymatous disease are above this value.

LEUCINE AMINOPEPTIDASE The analytical results obtained for leucine aminopeptidase activity in serum are represented in Table II. The highest

TABLE II

ANALYTICAL RESULTS FOR THE LEUCINE AMINOPEPTIDASE ACTIVITY

Diagnosis	*Mean Activity (μmol./h./ml.)*	*Standard Deviation*	*Range (max.-min.)*	*No. of Cases, Tests*
Normal	2·05	0·60	3·30-0·95	85, 102
Common duct stone (total)	7·20	2·25	12·15-4·50	17, 31
Common duct stone (max.)	8·25	2·40	12·15-5·10	17, 17
Cancer in head of pancreas	7·20	2·25	12·75-3·85	8, 15
Hepatic metastases	6·85	1·75	10·20-4·00	12, 18
Chronic hepatitis	4·50	1·90	8·35-0·75	22, 22
Epidemic hepatitis	4·35	1·80	8·10-1·65	6, 15
Uncomplicated cholecystolithiasis	1·80	0·75	3·30-0·50	8, 10
Complicated cholecystolithiasis	5·10	2·25	10·75-3·00	6, 14
Coronary occlusion, congested liver	5·55	1·75	9·80-3·75	9, 13
Coronary occlusion	2·05	0·60	3·15-0·75	28, 58

activities were encountered in patients with extra- and intrahepatic biliary obstruction, and there is no significant difference between the mean values obtained in cases of choledocholithiasis, carcinomata of the head of the pancreas, and liver metastases.

Moderately raised leucine aminopeptidase activity is observed in patients with acute (epidemic) hepatitis, chronic hepatitis, cholecystolithiasis complicated by cholecystitis and cholangitis, and in patients with myocardial infarction complicated by hepatic congestion. The mean values obtained in these diseases do not differ significantly; they are all significantly lower than the values obtained in patients with biliary obstruction.

The mean values obtained from the normal group, from patients with myocardial infarction without congested liver, and from patients with uncomplicated cholecystolithiasis did not display significant differences, but they are all significantly lower than the remaining mean values given.

In the normal group no distinction has been made with regard to sex, and the average age is high. It is therefore important that the mean value obtained from this group (2·05 μ mole/h./ml., SD = 0·60 μ mole) does not differ significantly from the value M = 2·40 μ mole/h./ml. (SD = 0·45 μ mole), obtained from 34 physically healthy men between 18 and 24 years of age.

In the 40 patients with intra- or extrahepatic biliary obstruction, in whom a certain discrepancy was observed between the raised alkaline phosphatase activity on the one hand and the normal glutamic oxalacetic transaminase activity and normal serum bilirubin concentration on the other, we find that the 71 analyses of leucine aminopeptidase activity all demonstrate pathological activity.

An attempt to find a value for serum leucine aminopeptidase activity which represents the borderline between values obtaining in parenchymatous liver diseases and those corresponding to choledocholithiasis, carcinoma of the head of the pancreas, and liver metastases gave the result that the value 5·40 μ mole/h./ml. divides the results in such a manner that 25% of 37 analyses from parenchymatous diseases lie above this value and 20% of 71 analyses from obstructive diseases lie below it.

A few patients with pathological serum leucine aminopeptidase values could not be included in any of the disease groups mentioned, *viz.*, patients suffering from malignant tumours in whom the presence of liver metastases had not been ascertained, patients with hepatic congestion resulting from a severe congestive heart failure, and patients with various hepatobiliary diseases (Table III).

TABLE III

LEUCINE AMINOPEPTIDASE, ALKALINE PHOSPHATASE, AND GLUTAMIC OXALACETIC TRANSAMINASE ACTIVITY IN SERUM IN HEPATO-BILIARY DISEASE

Diagnosis	*Leucine Amino-peptidase*	*Alkaline Phosphatase*	*Glutamic Oxalacetic Transaminase*
Chronic pancreatitis	6·30	34·8	0·3
Chronic pancreatitis	5·10	53·3	
Chlorpromazine jaundice	10·95	27·7	5·0
Infectious mononucleosis	11·70	47·6	16·9
Metastases to lymph nodes of porta hepatis	6·15	36·0	3·5

In post-operative bile obtained by T-tube from patients operated upon for cholelithiasis or diseases of the pancreas we have found leucine aminopeptidase activities up to four times as high as the highest serum activities measured. We also found that the leucine aminopeptidase activity in serum is independent of the function of the kidneys measured by the serum creatinine concentration.

DISCUSSION

Our results for glutamic oxalacetic transaminase activity in serum agree closely with the large number of available results reviewed by Wróblewski (1959). It appears from Table IV, as is stated elsewhere (Wróblewski and LaDue, 1955), that glutamic oxalacetic transaminase activity in serum is independent of the alkaline phosphatase activity. We are also unable to ascertain any correlation with serum leucine amonopeptidase activity. On the other hand, a comparison between leucine aminopeptidase and alkaline phosphatase activity in serum (Table IV and Fig. 1) shows considerable correlation. As in the case of alkaline phosphatase (Gutman, 1959; Gutman, Olson, Gutman, and Flood, 1940; Van der Meer, 1946), the highest serum leucine aminopeptidase activity is observed in biliary obstruction and lower activity in acute and chronic hepatitis and cholecystolithiasis complicated by cholecystitis and cholangitis. Our analytical results with regard to alkaline phosphatase activity in obstructive and parenchymatous liver diseases are distributed in the usual manner about the value 30 K.-A. units/100 ml.

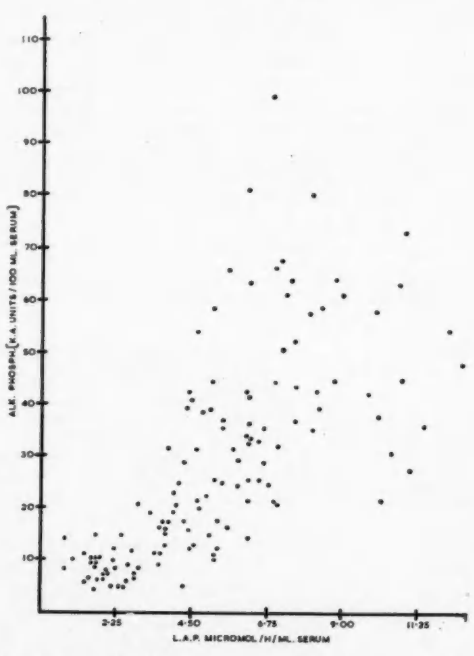

FIG. 1. *Relation between corresponding values of leucine aminopeptidase and alkaline phosphatase activity in serum from all patients suffering from hepato-biliary conditions.*

TABLE IV

MEANS OF ENZYMATIC ACTIVITY IN CLINICAL GROUPS OF PRESENT SERIES

Diagnosis	*Leucine Amino-peptidase (μmol./h./ml.)*	*Alkaline Phosphatase (K.-A. units)*	*GO-T. (units/ml.)*
Normal	2·05	8·0	1·1
Common duct stone (total)	7·20	43·3	4·8
Common duct stone (max.)	8·25	42·1	5·0
Cancer in the head of pancreas	7·20	56·5	4·9
Hepatic metastases	6·85	44·3	6·4
Chronic hepatitis	4·50	21·4	8·9
Epidemic hepatitis	4·35	21·0	16·9
Uncomplicated cholecystolithiasis	1·80	9·0	1·4
Complicated cholecystolithiasis	5·10	23·8	4·6
Coronary occlusion, congested liver	5·55		5·4
Coronary occlusion	2·05	8·8	7·0

(Gutman, 1959; Gutman *et al.*, 1940), and it seems as if a similar borderline value may be found for serum leucine aminopeptidase activity in these two disease groups, namely, 5·40 μ mole/h./ml.

In patients with extra- and intrahepatic biliary obstruction a dissociation between raised alkaline phosphatase activity and normal serum bilirubin concentration (Gutman *et al.*, 1940; Simons, 1954; Gibbons, 1957; Brem, 1955) has been described. In the present investigation this phenomenon applies uniformly to alkaline phosphatase as well as to leucine aminopeptidase activity. We find a similar dissociation between pathological activities of these two enzymes and normal glutamic oxalacetic transaminase activity (Table I).

Our results do not support the view held by Goldbarg and Rutenburg (1958) and Rutenburg *et al.* (1958) that the highest serum leucine aminopeptidase activity should indicate carcinoma of the pancreas. Unlike Arst *et al.* (1959), however, we are of the opinion that the determination of the leucine aminopeptidase activity in serum from jaundiced patients may be used to distinguish between parenchymatous and obstructive liver disease with the same reservations and the same uncertainty as those applying to alkaline phosphatase activity. In addition, raised leucine aminopeptidase activity may, as does a raised level of alkaline phosphatase activity (Gibbons, 1957), call attention to the possibility that a state of anicteric biliary obstruction exists. This view is supported by a recent work by Witts and Perry (1960). Likewise, Shay, Sun, and Siplet (1960) have recently concluded that raised serum leucine aminopeptidase activity must, as does raised alkaline phosphatase activity, be an indication of biliary obstruction.

Schwartz and Myers (1954) have shown that bilateral nephrectomy does not influence the peptidase activity of serum, but that the latter is reduced by its passage through the liver to such an extent that the arteriovenous deficit across the liver is proportional to the serum activity. They assume that these observations support the theory that serum peptidase is eliminated by the liver. Our observations (maximum serum activities in biliary obstruction, high enzymatic activity in bile) support the view of Schwartz and Myers, and suggest that the enzyme is eliminated by excretion in the bile.

CONCLUSION AND SUMMARY

In this comparative investigation of the clinical significance and diagnostic value of leucine aminopeptidase activity in serum, a pathologically raised level of activity has been observed in serum from patients with primary or secondary hepatobiliary diseases only. In these conditions the leucine aminoheptidase activity is closely related to that of the alkaline phosphatase in serum, with the same degree of elevation and the same relationship to the glutamic oxalacetic transaminase activity and bilirubin concentration in serum. The leucine aminopeptidase and alkaline phosphatase activities in serum are equivalent indicators of biliary obstruction but neither are of any value in differing between the causes of obstruction and both equally serve in differentiating obstructive from parenchymatous jaundice. Therefore, in the case of pancreatic cancer, a raised level of leucine aminopeptidase activity in serum must be considered due either to compression of the common bile duct by the primary tumour, to intrahepatic biliary obstruction caused by metastases to the liver, or to both.

REFERENCES

Adams, E., McFadden, M., and Smith, E. L. (1952). Peptidases of erythrocytes. I. Distribution in man and other species. *J. biol. Chem.*, **198**, 663-670.

Arst, H. E., Manning, R. T., and Delp, M. (1959). Serum leucine aminopeptidase activity: Findings in carcinoma of the pancreas, pregnancy and other diseases. *Amer. J. med. Sci.*, **238**, 598-609.

Braun-Falco, O., and Salfeld, K. (1957). Über das Verhalten der Leucin-Aminopeptidase-Activität im Blutserum und Blaseninhalt. *Arch. klin. exp. Derm.*, **205**, 103-111.

Brem, T. H. (1955). Recognition of biliary tract obstruction without jaundice. *J. Amer. med. Ass.*, **159**, 1624-1626.

Burstone, M. S., and Folk, J. E. (1956). Histochemical demonstration of aminopeptidase. *J. Histochem. Cytochem.*, **4**, 217-226.

Fleisher, G. A., and Butt, H. R. (1953). Peptidases in human serum: A comparison of activities between normal persons and patients with liver diseases and other pathological conditions. *J. clin. Invest.*, **32**, 674-680.

——, ——, and Huizenga, K. A. (1957). Enzymatic hydrolysis of l-leucylglycine in serum in hepatic disease. *Proc. Mayo Clin.*, **32**, 410-424.

Gibbons, T. B. (1957). Hyperphosphatasemia in patients without jaundice with hepatobiliary disease. *J. Amer. med. Ass.*, **164**, 22-28.

Goldbarg, J. A., and Rutenburg, A. M. (1958). The colorimetric determination of leucine aminopeptidase in urine and serum of normal subjects and patients with cancer and other diseases. *Cancer (Philad.)*, **11**, 283-291.

——, Pineda, E. P., and Rutenburg, A. M. (1959). The measurement of activity of leucine aminopeptidase in serum, urine, bile, and tissues. *Amer. J. clin. Path.*, **32**, 571-575.

Green, M. N., Kwan-Chung Tsou, Bressler, R., and Seligman, A. M. (1955). The colorimetric determination of leucine aminopeptidase activity with L-leucyl-β-naphthylamide hydrochloride. *Arch. Biochem.*, **57**, 458-474.

Gutman, A. B. (1959). Serum alkaline phosphatase activity in diseases of the skeletal and hepatobiliary systems. *Amer. J. Med.*, **27**, 875-901.

——, Olson, K. B., Gutman, E. B., and Flood, C. A. (1940). Effect of disease of the liver and biliary tract upon the phosphatase activity of the serum. *J. clin. Invest.*, **19**, 129-152.

Henley, K. S., and Pollard, H. M. (1955). A new method for the determination of glutamic oxalacetic and glutamic pyruvic transaminase in plasma. *J. Lab. clin. Med.*, **46**, 785-789.

Hill, R. L., and Smith, E. L. (1957). Leucine aminopeptidase. VII. Action on long chain polypeptides and proteins. *J. biol. Chem.*, **228**, 577-600.

Kind, P. R. N., and King, E. J. (1954). Estimation of plasma phosphatase by determination of hydrolysed phenol with aminoantipyrine. *J. clin. Path.*, **7**, 322-326.

Kowlessar, O. D., Haeffner, L. J., and Sleisenger, M. H. (1960). Localization of leucine aminopeptidase in serum and body fluids by starch gel electrophoresis. *J. clin. Invest.*, **39**, 671-675.

Pearse, A. G. E., and Tremblay, G. (1958). Leucine aminopeptidase in rat parathyroid and its relation to parathyroid hormone production. *Nature (Lond.)*, **181**, 1532-1533.

Rutenburg, A. M., Goldbarg, J. A., and Pineda, E. P. (1958). Leucine aminopeptidase activity. Observations in patients with cancer of the pancreas and other diseases. *New Engl. J. Med.*, **259**, 469-472.

Shay, H., Sun, D. C. H., and Siplet, H. (1960). Leucine aminopeptidase: Significance of serum elevations in disease of the hepatobiliary-pancreatic system. *Amer. J. dig. Dis.*, **5**, 217-232.

Schwartz, T. B., and Myers, J. D. (1954). Observations on the visceral removal of plasma peptidase activity. *J. clin. Invest.*, **33**, 337-341.

Simons, R. L. (1954). Hepatic tests in metastatic carcinoma of the liver. A review of seventy-three cases proven at necropsy. *Amer. J. med. Sci.*, **228**, 312-316.

Smith, E. L. (1951). The specificity of certain peptidases. II. Leucine aminopeptidase. *Advanc. Enzymol.*, **12**, 196-206.

——, and Spackman, D. H. (1955). Leucine aminopeptidase. V. Activation, specificity, and mechanism of action. *J. biol. Chem.*, **212**, 271-299.

Van der Meer, P. (1946). The phosphatase content of the serum in jaundice. *Acta med. scand.*, **126**, 265-272.

Willighagen, R. G. J., and Planteydt, H. T. (1959). Aminopeptidase activity in cancer cells. *Nature (Lond.)*, **183**, 263-264.

Witts, L. J., and Perry, S. W. (1960). Leucine aminopeptidase with special reference to ulcerative colitis. *Gut*, **1**, 140-141.

Wróblewski, F. (1959). The clinical significance of transaminase activities of serum. *Amer. J. Med.*, **27**, 911-923.

——, and LaDue, J. S. (1955). Serum glutamic-oxalacetic-transaminase activity as an index of liver-cell injury from cancer. *Cancer (Philad.)*, **8**, 1155-1163.

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The effect of anticholinergic drugs on the electrolyte content of gastric juice

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SYNOPSIS Gastric secretion was stimulated by insulin hypoglycaemia and the effect of increasing doses of anticholinergic drugs on the volume, acid, and electrolyte content of gastric juice was studied. The sodium and potassium output was depressed to a far less extent than the acid output and the drop in volume of secretion and acid output after anticholinergic drugs is almost entirely due to decreased acid secretion. With increasing anticholinergic suppression of the volume of secretion, there is a marked fall in potassium output and a lesser fall in sodium output. The potassium concentration showed a slight fall and there was a rise in sodium concentration.

The effect of various anticholinergic drugs on gastric acid secretion has been studied by many workers. The acid concentration and output so investigated represents the amount of parietal secretion remaining after dilution and partial neutralization by the alkaline component, chiefly mucus and sodium and potassium chloride and bicarbonate. Hence it is of some interest to know the effect of anticholinergic agents on the electrolyte content of gastric juice; as far as can be ascertained, this has not been previously studied.

METHODS

The patients studied were psychiatric patients having insulin coma therapy. All were free of gastrointestinal disease. Their repeated treatment enabled many observations to be made on the same patient. A Cleland tube was passed when the patient became comatose, coma being considered to be present when patients no longer responded to painful stimuli and bilateral extensor plantar responses were present. Continuous suction was applied for about 15 minutes to remove the gastric residue and two 15-minute specimens were collected during the last 30 to 45 minutes spent in coma. Blood sugar estimations were done initially but were discontinued when it was found reproducible control results were obtained under the above conditions, whatever the actual blood sugar level found. Patients were included in this study only if the control secretory rates were high (>3 ml./min.) and the specimens were not bile-stained.

The series of anticholinergic drugs included atropine, propantheline, oxyphencyclimine, and propionyl atropine methyl nitrate. These were administered orally two to two and a half hours before the onset of coma.

From the design of the experiments, maximal hypoglycaemic stimulation was considered to be present because the patients were comatose from hypoglycaemia during the period gastric secretion was collected. That this is a constant base line from which to assess changes is shown by the fact, discussed later in this article, that in 10 control observations on three patients the standard deviation was only 3·7% of the mean secretion under these circumstances. A greater error than this could be expected, however, when the volume of secretion was small. It is appreciated that the time of maximum action of the drugs administered varied and would not necessarily coincide with the time of collection of gastric juice. It is seen in Table I, however, that the control secretions were relatively constant for each patient and it is reasonable to assume that the change after the drugs was due to the anticholinergic drug administered. The different responses after drugs were a manifestation of different pharmacological effects related to the potency of the drug, its dose, and the time relationship of the time of collection to the time of its maximal action. All that is of importance in this investigation is that the changes in secretion in the studies after the drugs are due to the drug alone and not to other factors. Which of the four drugs was used is irrelevant in view of the information sought.

The acid concentration was estimated using phenol red as indicator. Schales and Schales method was used in the estimation of chloride. The sodium and potassium concentrations were determined using an Eel flame photometer.

RESULTS

The results from the three patients studied are given in Table I; the findings from one patient are represented graphically in Figure 1. The findings

TABLE I

EFFECT OF ANTICHOLINERGIC DRUGS ON ACID, CHLORIDE, SODIUM AND POTASSIUM CONCENTRATION AND OUTPUT

Patient	*Vol.* (*ml.*)	*H+*		*Cl−*		*Na+*		*K+*	
		Concentration (*mEq./l.*)	*Output* (*mEq.*)	*Concentration* (*mEq./l.*)	*Output* (*mEq.*)	*Concentration* (*mEq./l.*)	*Output* (*mEq.*)	*Contentration* (*mEq./l.*)	*Output* (*mEq.*)
1, control	184	92	17·0	133	27·5	29	5·34	15·7	2·90
	181	95	17·2	140	25·4	33	5·97	13·4	2·43
	173	108	18·7	143	24·4	20	3·46	16·0	2·77
After anticholinergic drugs	164	103	16·9	146	24·1	25	4·10	16·4	2·69
	156	95	14·8	135	21·0	25	3·91	13·0	2·03
	137	89	12·2	136	18·6	30	4·15	13·5	1·85
	91	90	8·2	145	13·2	34	3·09	15·9	1·45
	82	83	7·3	134	10·9	36	2·93	13·4	1·10
	80	107	8·5	139	11·1	18	1·48	14·7	1·17
	62	86	5·3	136	8·4	39	2·42	11·8	0·74
	52	72	3·9	113	5·9	38	1·92	12·7	0·66
	39	86	3·4	142	5·6	39	1·53	9·4	0·36
	34	59	2·2	116	4·0	47	1·62	10·0	0·34
2, control	91	96	8·7	154	14·0	43	5·81	12·1	1·10
	88	99	8·7	166	14·6	43	3·78	10·0	0·88
	88	95	8·4	149	13·0	45	3·96	11·2	0·98
	73	99	7·2	150	11·0	38	2·77	12·7	0·93
After anticholinergic drugs	58	60	3·5	128	7·4	50	2·90	12·1	0·70
	28	44	1·2	137	3·8	87	2·44	9·0	0·26
	8	17	0·2	107	0·8	91	0·73	5·0	0·07
3, control	119	100	12·0	149	17·8	30	3·63	14·5	1·72
	98	98	9·6	151	14·8	38	3·74	16·8	1·65
After anticholinergic drugs	99	71	7·0	133	13·2	42	4·20	14·6	1·44
	72	77	5·5	138	9·9	51	3·67	13·6	0·98

[1]The results are listed in order of decreasing volume. In the actual experiment the control studies and the studies after drugs were randomized. The volume secreted and the outputs are expressions of secretion during 30 minutes.

are listed in order of decreasing volume of secretion, irrespective of the anticholinergic agent used. The output represents the output during 30 minutes of the hour each patient spent in coma.

Summarizing these results, (1) there was a progressive drop in acid output with decreasing volume of secretion; the acid concentration showed a less marked fall. (2) The sodium and potassium output fell, the potassium output falling to a greater extent than the sodium output. The potassium concentration remained relatively fixed or showed a slight fall with decreasing volume of secretion while the sodium concentration rose.

DISCUSSION

Previous studies have shown that despite the fact that anticholinergic drugs will inhibit the output of acid, the concentration of acid shows relatively little fall (Keeton, Luckhardt, and Koch, 1920; Polland, 1930; Gray, 1943; Notkin, Forse, and Webster, 1953; Piper, Elliott, Sietsma, and Pryor, 1960). It is possible that the drop in acid secretion could be accompanied by a fall in the output of the alkaline secretion, or the latter could remain relatively unchanged. Like the acid component, sodium is secreted at a concentration approximately isotonic with plasma (Hollander, 1952; Grossman, 1960) but Grossman (1960) has shown that the secretion of the pyloric antrum, the chief source of the alkaline component, is not influenced by the various drugs that increase gastric secretion. It has also been demonstrated that the sodium output following histamine stimulation varies slightly from individual to individual, compared with the marked variation in acid output found in different individuals (Piper, 1960).

The changes observed here in the concentration of sodium and potassium are consistent with the observed changes in the latter cations with variation in the volume and acid output after histamine. Sodium concentration is inversely related to the acid concentration (Hollander, 1952; Gray, Bucher, and Harman, 1941; Riddell, Strong, and Cameron, 1960) and there is a slight drop in potassium concentration at low volume secretory rates (Riddell *et al.*, 1960). The output responses to increasing doses of anticholinergic drugs show that the latter influences the alkaline component to a lesser extent than the acid secretion and the change in volume after anti-

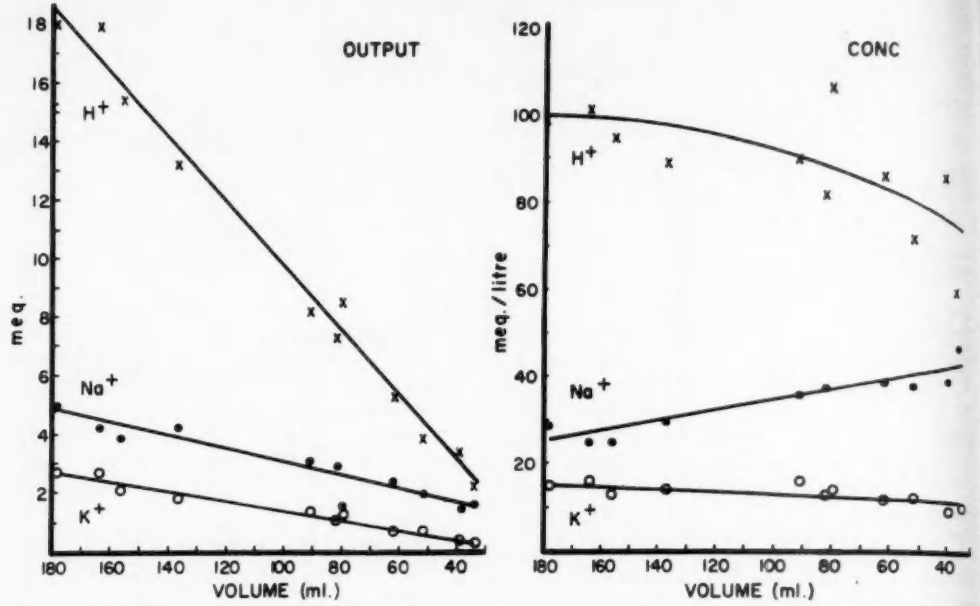

FIG. 1. *Graph showing effect of anticholinergic drugs on the electrolyte output and concentration in gastric juice of one patient (Patient I).*

cholinergic drugs is almost entirely due to diminished acid secretion. It is seen that in Patient 1 (Table I) the drop in the acid output from 17·0 mEq. to 2·2 mEq. was accompanied by a drop in the sodium output from 5·42 mEq. to 1·62 mEq. and the potassium output from 2·90 mEq. to 0·34 mEq. The greater drop in potassium output compared with the sodium output is due to the fact that the concentrations of these cations vary in opposite directions when plotted against the volume of secretion; this suggests that they are secreted by different mechanisms. The drop in acid concentration with low acid secretory rates is no doubt due to neutralization and dilution of the acid secretion by the alkaline component.

In studies such as these, salivary contamination and incomplete collection are a possible source of error. In the present study, because of the use of anticholinergic agents, salivary contamination was least where the volumes of secretion were small and where the effect of contamination would be greatest. It is felt that the error due to the incomplete collection was small; in 10 control observations done on three patients, the coefficient of variation was only 3·7%. A greater error could be expected, however, when the volumes of secretion were small.

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REFERENCES

Gray, J. S. (1943). Physiology of parietal cell with special reference to formation of acid. *Gastroenterology*, **1**, 390-400.

——, Bucher, G. R., and Harman, H. H. (1941). The relationship between total acid and neutral chlorides of gastric juice. *Amer. J. Physiol.*, **132**, 504-516.

Grossman, M. I. (1960). The pyloric gland area of the stomach. *Gastroenterology*, **38**, 1-6.

Hollander, F. (1952). Symposium on secretion of electrolytes; gastric secretion of electrolytes. *Fed. Proc.*, **11**, 706-714.

Keeton, R. W., Luckhardt, A. B., and Koch, F. C. (1920). Gastrin Studies IV. The response of the stomach mucosa to food and gastric bodies as influenced by atropine. *Amer. J. Physiol.*, **51**, 469-483.

Notkin, L. J., Forse, R. A., and Webster, D. R. (1953). Effect of gastric secretory depressants (banthine, bellafoline, amidopyrine) on innervated gastric pouch in dogs. *Amer. J. dig. Dis.*, **20**, 277-284.

Piper, D. W. (1960). Thesis submitted for Doctorate of Medicine, University of Sydney.

——, Elliott, F. M., Sietsma, A. S., and Pryor, A. W. (1960). The effect of a new anticholinergic agent, oxyphencyclimine hydrochloride, on gastric secretion. *Med. J. Aust.*, **1**, 236-240.

Polland, W. S. (1930). Effect of atropine upon gastric secretion after histamine stimulation. *J. clin. Invest.*, **9**, 319-332.

Riddell, M. J., Strong, J. A., and Cameron, D. (1960). The electrolyte concentration of human gastric secretion. *Quart. J. exp. Physiol.*, **45**, 1-11.

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Non-esterified fatty acids and lipoprotein lipase activity in patients with cirrhosis of the liver

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SYNOPSIS After the intravenous injection of heparin the plasma concentration of non-esterified fatty acids rises in normal subjects and in patients with nephrosis or diabetes but not in patients with portal cirrhosis.

In 1943, Hahn observed that when blood to which heparin had been added as an anticoagulant was infused into lipaemic dogs the lipaemia cleared very rapidly. When heparin was added *in vitro* the lipaemia remained unaltered. It is now accepted that this clearing of the lipaemia after the injection of heparin is due to the appearance in the blood stream of a lipase (lipoprotein lipase) which catalyses the hydrolysis of the triglycerides contained in chylomicrons and low-density lipoproteins into glycerol and non-esterified fatty acids. Thus, after the intravenous injection of heparin, there is a rise in the concentration of non-esterified fatty acids in the plasma (Grossman, Palm, Becker, and Moeller, 1954; Gordon and Cherkes, 1956). The non-esterified fatty acids are 'free' fatty acids in the sense that they are not in covalent linkage although they are very tightly bound to albumin. Albumin, therefore, stimulates the lipolytic process by removing the fatty acids which are produced. It seems likely that heparin acts by virtue of its highly electro-negative sulphate groups, mobilizing an apo-enzyme from the tissues where it is normally present and effecting its rapid attachment to the substrate. The active enzyme is a union of heparin and apo-enzyme.

In the absence of a previous injection of heparin, the lipoprotein lipase activity of normal plasma is small or absent as measured *in vitro* by the reduction of turbidity (Engelberg, 1956a and 1958) and by the increase in the concentration of non-esterified fatty acids (Gates and Gordon, 1958) or glycerol (Cleland and Iacono, 1957). Baker, Levine, Turner, and Dubin (1958) reported that after the intravenous injection of a small (3 mg.) amount of heparin the clearing activity *in vitro* in the plasma of patients with Laennec's cirrhosis was greater than in normals. The dose of heparin used by these authors was chosen on the basis of a previous observation (Block, Mann, and Barker, 1951) that a dose of 5 mg. of heparin brings about a complete clearing of the lipaemic plasma whereas 3 mg. 'just fails to restore completely the translucency of plasma' during alimentary lipaemia. Patients with Laennec's cirrhosis and a low serum albumin level showed a maximum clearing. This was in contrast to the plasma of a nephrotic patient with hypoalbuminaemia which showed no clearing activity, an observation in keeping with the findings of Herzstein, Wang, and Aldersberg (1954). The animal experiments by Rosenman and his colleagues (Rosenman, Friedman, and Byers, 1956; Rosenman and Friedman, 1957; Rosenman and Byers, 1959) have suggested that the reduced clearing activity of the nephrotic plasma is partly attributable to the low levels of albumin which fail to remove the excess of fatty acids produced during lipolysis. The different effect of a low serum albumin level in these two different diseases is paradoxical, and becomes more so if one bears in mind that patients with cirrhosis of the liver have high levels of plasma non-esterified fatty acids (Stormont and Mackie, 1959; Wajchenberg, Hoxter, Mello, and Ulhoa Cintra, 1960), and consequently an even more reduced 'potential' amount of albumin to serve as an acceptor of the fatty acids released during lipolysis.

In the present work the lipolytic effect *in vivo* of intravenous heparin has been followed by measuring the plasma concentration of non-esterified fatty acids. Normal subjects and patients with cirrhosis of the liver were studied. The effect of heparin *in vivo* was also followed in three patients with nephrosis and in two patients with diabetes mellitus. In addition, the endogenous lipolytic activity in the plasma of cirrhotic patients has been measured *in vitro* and compared with that of normal plasma.

MATERIAL AND METHODS

Ten patients with portal cirrhosis and two patients with biliary cirrhosis were studied. The diagnosis had been confirmed histologically in each case. The clinical status and liver function tests were compatible with the histological diagnosis in all of them. Further studies were made on 11 normal subjects, on two patients with diabetes mellitus, and on three patients with a low plasma albumin level associated with nephrosis.

The concentration of non-esterified fatty acids in plasma was estimated by Gordon, Cherkes, and Gates' method (1957). The composition of non-esterified fatty acids extracted from human plasma by this method has been studied by means of a Pye Argon chromatograph (Chlouverakis and Harris, 1960) and found to be the same in cirrhotic patients as it is in normals.

EFFECT OF HEPARIN *IN VIVO* For this purpose, heparin was injected into a peripheral vein and its effect on the concentration of non-esterified fatty acids measured in arterial blood. Arterial blood was chosen since peripheral venous levels of non-esterified fatty acids are largely dependent on local metabolism (Gordon *et al.*, 1957). Two groups of patients were studied, one with, and the other without, cirrhosis. In each group, studies were made of the effect of heparin both under fasting conditions and three hours after a fatty meal.

Subjects without cirrhosis The effect of heparin was studied under fasting conditions in three normal subjects, in two patients with diabetes, and in one patient with a low serum albumin level (2·3 g./100 ml.) associated with nephrosis. The subjects had fasted overnight for 12 hours. An indwelling arterial needle was inserted and two samples of blood drawn into heparinized syringes at an interval of five minutes. After the second sample was taken, 100 mg. of sodium heparin was injected into a peripheral vein and further samples of arterial blood taken after 15, 30, 60, and 90 minutes. The dose of 100 mg. of heparin was chosen in order to produce a maximum effect.

In a control group of five normal subjects, the same procedure was undertaken without the injection of heparin. Its purpose was to exclude the possibility that any rise of the non-esterified fatty acid level after heparin was due to continued starvation during the period of sampling.

In three normal subjects and two patients with a low serum albumin level (2·0 and 2·0 g./100 ml.) due to nephrosis a similar study was made after the administration of a fatty meal. The subjects fasted overnight. In the morning they were given 100 g. of milk cream. Three hours later an arterial needle was inserted and two samples of blood withdrawn into heparinized syringes. Then 100 mg. of sodium heparin was injected intravenously and further samples of arterial blood taken after 10, 20, 40, and 60 minutes.

Patients with cirrhosis The effect of heparin was studied under fasting conditions in five patients with portal cirrhosis and in two patients with biliary cirrhosis. The procedure was the same as that used in the subjects without cirrhosis.

In four patients with portal cirrhosis and one patient with biliary cirrhosis, the effect of heparin was studied after a fatty meal by the same method.

ENDOGENOUS LIPOLYTIC ACTIVITY *IN VITRO* The endogenous lipolytic activity was studied in the plasma of four normal subjects and five patients with portal cirrhosis. Venous blood (20 ml.) was drawn into a heparinized syringe after 12 hours' starvation overnight. The plasma from this blood was divided into six portions, each of 1 ml. The first portion was used for the estimation of non-esterified fatty acids. The other five portions were each placed in a test-tube. Nothing was added to the first tube. To the remaining four test-tubes was added respectively 0·1, 0·2, 0·3, and 0·4 ml. of an 0·2% freshly prepared emulsion of cocoanut oil. All tubes were then covered and placed in a water-bath at 37°C. for two hours. Estimations of the concentration of non-esterified fatty acids were then made on the contents of each tube.

RESULTS

The concentration of non-esterified fatty acids was 1,470 ± 312 μEq./l. (mean ± S.D.) in the arterial blood of five fasting patients with portal cirrhosis which compares with 712 ± 134 μEq./l. found in this laboratory in the arterial blood of normal subjects.

After the injection of 100 mg. of heparin the concentration of non-esterified fatty acids in the plasma rose appreciably (Fig. 1) in all the fasting subjects without cirrhosis, including the two patients with diabetes and one with nephrosis. The rise in the diabetic patients appeared to be greater than in the normals. Fig. 1 also shows the concentration of non-esterified fatty acids during the same period of time in the control group of fasting normal subjects who did not receive heparin.

Four of the five fasting patients with portal cirrhosis showed no significant change in the plasma non-esterified fatty acid concentration after the injection of heparin. One patient showed a fall. Two fasting patients with biliary cirrhosis showed a small rise (Fig. 2).

The response to heparin three hours after a fatty meal in the subjects without cirrhosis is shown in Fig. 3. In all the level of non-esterified fatty acids rose, the average of the maximal rise being 1,804 μEq./l. This response was most marked in the two nephrotic patients.

Fig. 4 shows the effect of heparin during an alimentary lipaemia in patients with cirrhosis of the liver. Two patients with portal cirrhosis showed a rise in the concentration of non-esterified fatty acids similar to that found in normals. One patient showed a fall and another a very slight rise. The only patient with biliary cirrhosis who was studied in this fashion responded by a slight rise.

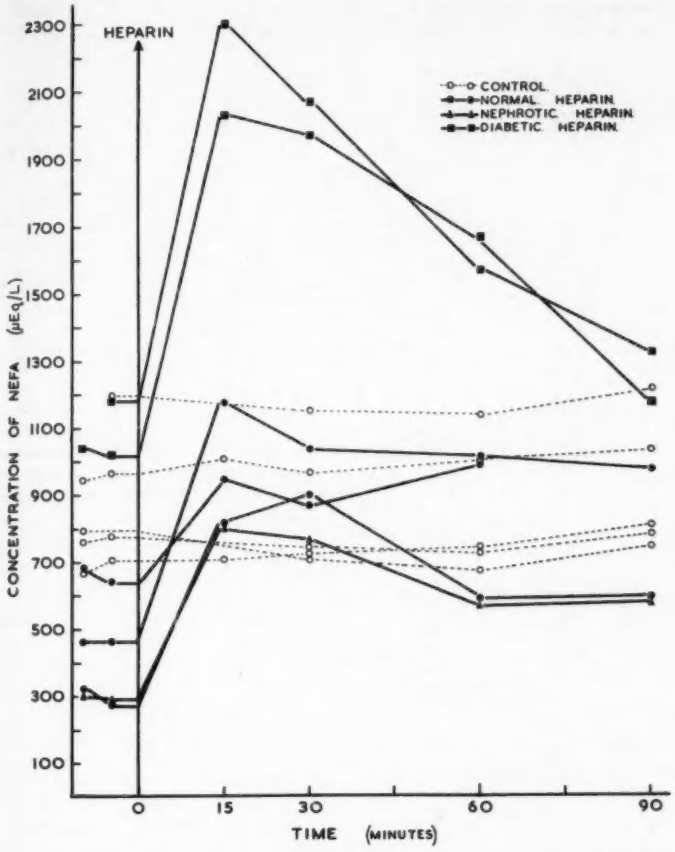

FIG. 1. *The effect of intravenous heparin on the plasma concentration of non-esterified fatty acids of fasting, non-cirrhotic subjects.*

Fig. 5 shows the results of the estimation *in vitro* of endogenous plasma lipolytic activity on four normal subjects and five patients with portal cirrhosis. None of the normal subjects showed any lipolytic activity. On the other hand, only one of the five cirrhotic patients did not show enzyme activity. The other four showed a substantial activity which varied with the different concentrations of the exogenously added substrate.

DISCUSSION

The central finding of the studies *in vivo* is that, in the fasting state, heparin causes a rise in the plasma concentration of non-esterified fatty acids in normal people but no rise in patients with portal cirrhosis. The cause of this lack of response on the part of the cirrhotic patients does not appear to be a lack of plasma albumin, since a nephrotic patient with a low serum albumin level responded normally. Moreover, in two of the cirrhotic patients, the serum albumin had been restored to normal levels by means of infusions of human albumin before the heparin test was undertaken.

In diabetic patients who had a high level of plasma non-esterified fatty acids the response to heparin appeared to be greater than normal, so that the lack of response in the cirrhotic patients is not due to the high initial levels of plasma non-esterified fatty acids, which might occupy most of the available sites for the attachment of the fatty acids in the albumin molecule.

A further possible reason for the difference between cirrhotics and normals could be a relatively low concentration of available lipid substrate (Baker *et al.*, 1958). When this possibility was checked by

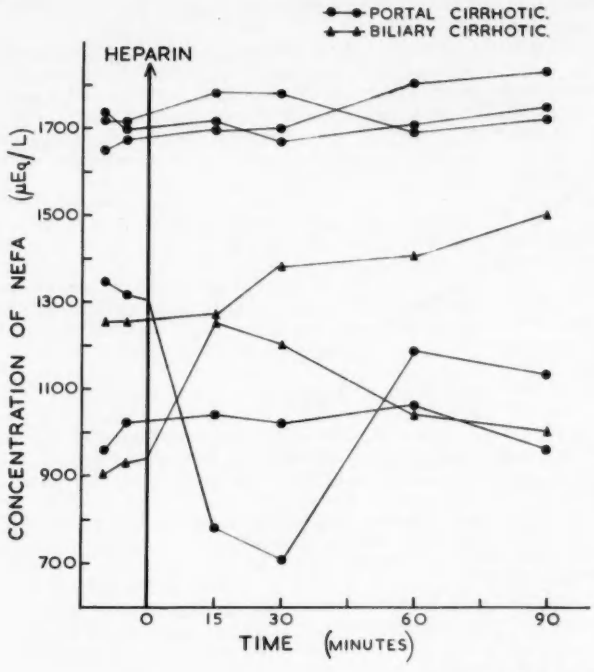

FIG. 2. *The effect of intravenous heparin on the plasma concentration of non-esterified fatty acids of fasting patients with cirrhosis.*

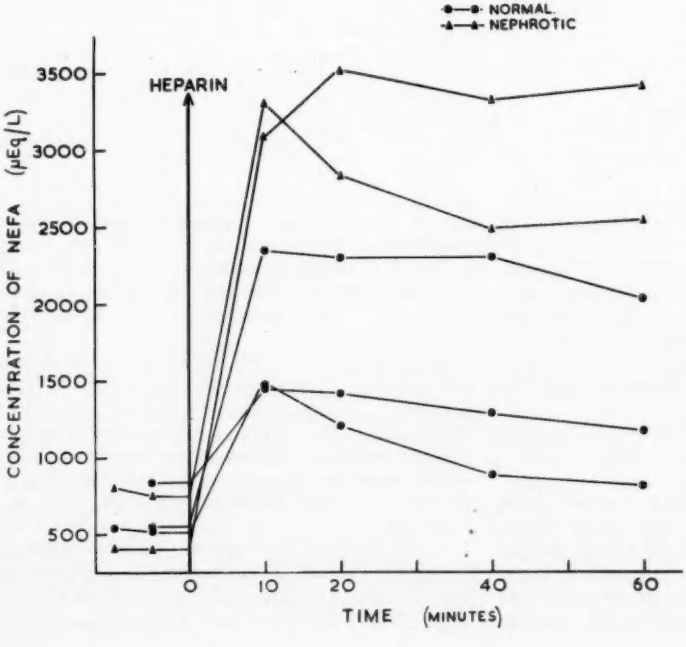

FIG. 3. *The effect of intravenous heparin on the plasma concentration of non-esterified fatty acids of non-cirrhotic subjects in a state of alimentary lipaemia.*

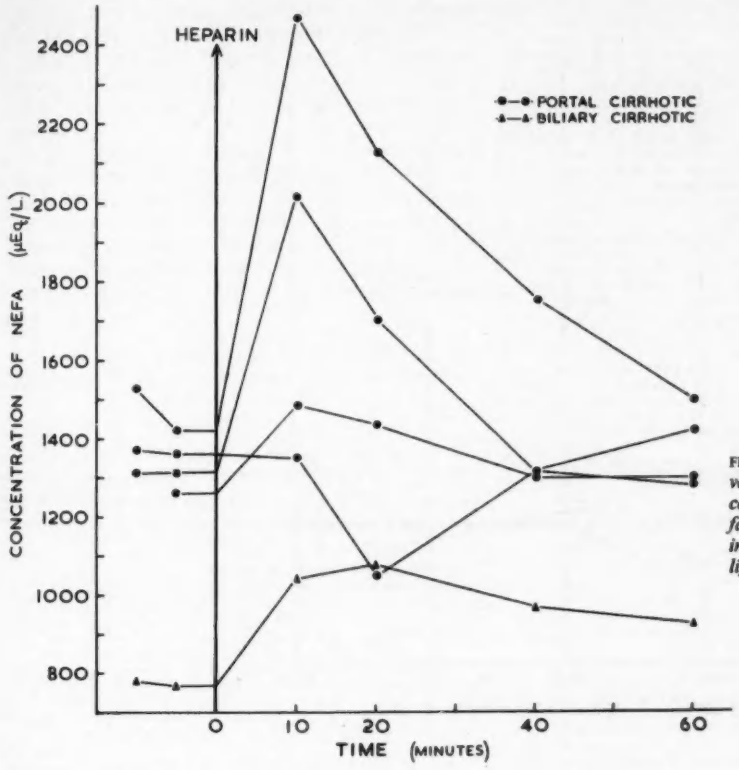

FIG. 4. *The effect of intravenous heparin on the plasma concentration of non-esterified fatty acids of cirrhotic patients in a state of alimentary lipaemia.*

inducing an alimentary lipaemia, it was found that two of the four cirrhotic patients studied had a normal response. This would suggest that although a relatively low level of substrate can be an important factor it does not explain the lack of response to heparin in every cirrhotic patient.

It is conceivable that inhibitors to lipoprotein lipase may be present in the blood of cirrhotic patients. The presence of such substances seems, however, highly unlikely in view of the increased lipolytic activity found in the plasma of most cirrhotics during the present study. This increased activity can be explained by the observation that the liver inactivates lipoprotein lipase (Jeffries, 1954; Spitzer and Spitzer, 1956). Since the liver may be responsible for the destruction or de-activation of heparin (Jaques, 1940; Jaques and Keeri-Szanto, 1952), which is an integral part of the lipoprotein lipase molecule, it is possible that the inactivation of the lipolytic enzyme by the liver may be merely the result of the destruction of heparin. This would result in the presence of increased amounts of heparin in the blood of patients with hepatic damage. Such a prolonged hyperheparinaemia might exhaust the tissue stores of apo-enzyme (Korn, 1955) which could account for the lack of a substantial rise in the plasma non-esterified fatty acids after heparin in two of the four cirrhotic patients in whom an adequate concentration of lipid substrate had been assured by the production of an alimentary lipaemia.

There is also evidence that an excess of heparin can inactivate lipoprotein lipase activity (Korn, 1955; Hollett and Meng, 1956a) and this might explain the fall in the concentration of non-esterified fatty acids which was observed in one cirrhotic patient both under starving and lipaemic conditions.

The high concentration of non-esterified fatty acids in the blood of patients with portal cirrhosis confirms the findings of Stormont and Mackie (1959) and Wajchenberg *et al.* (1960). These high levels could be explained by the increased endogenous lipolytic activity of the plasma found in the present

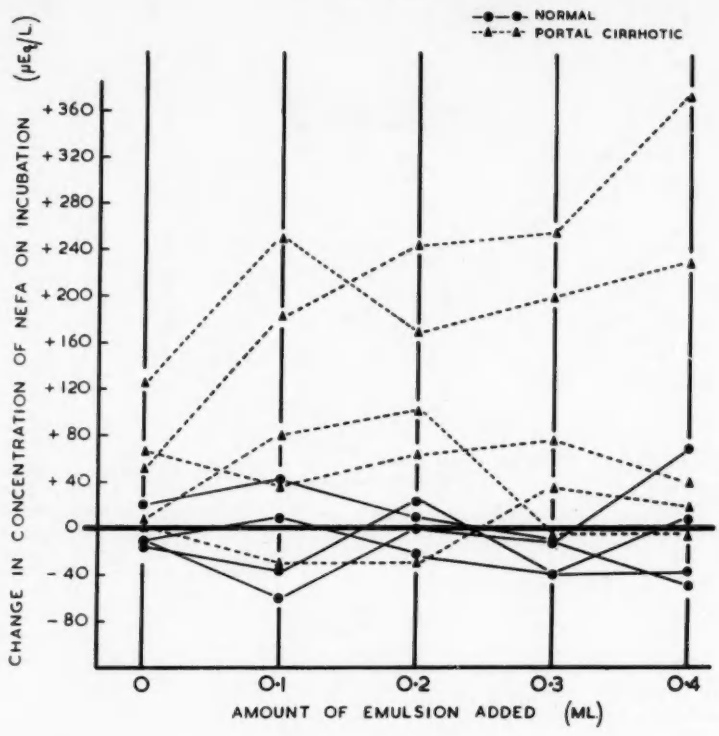

FIG. 5. *The lipolytic activity* in vitro *of normal plasma and of plasma from cirrhotic patients.*

study. It is of interest that the only cirrhotic patient who did not show lipolytic activity had a normal concentration of non-esterified fatty acids (730 μEq./l.). Wajchenberg and his colleagues suggested that the high levels of plasma non-esterified fatty acids in cirrhotics are due to a failure of the damaged liver to withdraw non-esterified fatty acids from the circulation. This suggestion, however, takes no account of the fact that non-esterified fatty acids are also widely extracted and oxidized by other tissues (Gordon and Cherkes, 1956; Gordon *et al.*, 1957; Spitzer and Roheim, 1958; Carlson and Pernow, 1959).

Hollet and Meng (1956b) reported that after an intravenous injection of heparin the lipolytic activity *in vitro* of the plasma was less in diabetics than in normals as measured by a decrease in optical density. Herzstein *et al.* (1954) made a similar observation in nephrotic patients. In the present study, heparin caused a greater rise in plasma non-esterified fatty acids in diabetics and nephrotics than it did in normals. This apparent discrepancy seems to be due to the fact that lipolysis can occur in the absence of any decrease in optical density (Engelberg, 1956b). This is supported by the observation of Herzstein and his co-workers that in all but one of their nephrotic patients there was a decrease in the plasma neutral fat after heparin and by our observation that no visual clearing of the plasma occurred in the two nephrotic patients who showed a great release of non-esterified fatty acids. It seems that the levels of the serum albumin encountered even in patients with severe hypoalbuminaemia do not affect the rate of the lipolytic reaction, a view which is in keeping with that of Baker (1957).

SUMMARY

The intravenous injection of heparin under fasting conditions causes a rise in plasma non-esterified fatty acids in normal subjects and in patients with nephrosis or diabetes. The rise is higher if heparin is given in the presence of an alimentary lipaemia.

In fasting patients with portal cirrhosis, the plasma non-esterified fatty acids fail to rise after heparin and may even fall. In cirrhotic patients with

an alimentary lipaemia, the response of the plasma non-esterified fatty acids to heparin is variable.

No endogenous lipolytic activity could be demonstrated in normal plasma, but it was observed in the plasma of four out of five patients with portal cirrhosis.

It is suggested that the high levels of plasma non-esterified fatty acids found in cirrhotic patients are due to the increased lipolytic activity of the plasma. The lack of effect of heparin in fasting cirrhotic patients is thought to be due partly to a relative deficiency of lipid substrate in the plasma and partly to exhaustion of the stores of lipolytic apo-enzyme in the tissues.

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REFERENCES

Baker, S. P. (1957). Heparin-activated clearing factor. Standardized test, agewise application, and clinical observations. *Circulation*, **15**, 889-896.

——, Levine, H., Turner, L., and Dubin, A. (1958). Lipoprotein lipase response in Laennec's cirrhosis. *Proc. Soc. exp. Biol. (N.Y.)*, **99**, 670-672.

Block, W. J. Jr., Mann, F. D., and Barker, N. W. (1951). Effect of small doses of heparin in increasing the translucence of plasma during alimentary lipemia: studies in normal individuals and patients with atherosclerosis. *Proc. Mayo Clin.*, **26**, 246-249.

Carlson, L. A., and Pernow, B. (1959). Studies on blood lipids during exercise. I: arterial and venous plasma concentrations of unesterified fatty acids. *J. Lab. clin. Med.*, **53**, 833-841.

Chlouverakis, C., and Harris, P. (1960). Composition of the free fatty acid fraction in the plasma of human arterial blood. *Nature (Lond.)*, **188**, 1111-1112.

Cleland, W. W., and Iacono, J. M. (1957). Role of lipoprotein lipase in intravascular clearing of fat in human subjects. *Fed. Proc.*, **16**, 383.

Engelberg, H. (1956a). Human endogenous lipemia clearing activity. Studies of lipolysis and effects of inhibitors. *J. biol. Chem.*, **222**, 601-610.

—— (1956b). *Studies of circulating human endogenous lipoprotein lipase (heparin lipaemia clearing factor)*. Third Int. Conf. on Biochem. Problems of Lipids, pp. 367-378.

—— (1958). Human endogenous plasma lipemia clearing activity. Observations in 482 individuals. *J. appl. Physiol.*, **13**, 375-380.

Gates, H. S. Jr., and Gordon, R. S. Jr. (1958). Demonstration of lipoprotein lipase in fasting human serum. *Fed. Proc.*, **17**, 437.

Gordon, R. S. Jr., and Cherkes, A. (1956). Unesterified fatty acid in human blood plasma. *J. clin. Invest.*, **35**, 206-212.

——, ——, and Gates, H. (1957). Unesterified fatty acid in human blood plasma. II: The transport function of unesterified fatty acid. *Ibid.*, **36**, 810-815.

Grossman, M. I., Palm, L., Becker, G. H., and Moeller, H. C. (1954). Effect of lipemia and heparin on free fatty acid content of rat plasma. *Proc. Soc. exp. Biol. (N.Y.)*, **87**, 312-315.

Hahn, P. F. (1943). Abolishment of alimentary lipemia following injection of heparin. *Science*, **98**, 19-20.

Herzstein, J., Chun-I Wang, and Adlersberg, D. (1954). Effect of heparin on plasma lipid partition in man: studies in normal persons and in patients with coronary atherosclerosis, nephrosis and primary hyperlipemia. *Ann. intern. Med.*, **40**, 290-306.

Hollett, C., and Meng, H. C. (1956a). Comparison of fat emulsion clearing activities of postheparin plasma and pancreatic lipase. *Amer. J. Physiol.*, **184**, 428-432.

——, —— (1956b). *Purification and characterization of a lipaemic clearing factor inhibitor from normal plasma*. Third Int. Conf. on Biochem. Problems of Lipids, pp. 190-199.

Jaques, L. B. (1940). Heparinase. *J. biol. Chem.*, **133**, 445-451.

——, and Keeri-Szanto, E. (1952). Heparinase. II. Distribution of enzyme in various tissues and its action on natural heparins and certain synthetic anticoagulants. *Canad. J. med. Sci.*, **30**, 353-359.

Jeffries, G. H. (1954). The sites at which plasma clearing activity is produced and destroyed in the rat. *Quart. J. exp. Physiol.*, **39**, 261-270.

Korn, E. D. (1955). Clearing factor, a heparin-activated lipoprotein lipase. I. Isolation and characterization of the enzyme from normal rat heart. *J. biol. Chem.*, **215**, 1-14.

Rosenman, R. H., and Byers, S. O. (1959). Study of possible causal role of lipoprotein lipase deficiency in nephrotic hyperlipemia. *J. clin. Invest.*, **38**, 1036.

——, and Friedman, M. (1957). *In vivo* studies of the role of albumin in endogenous and heparin-activated lipemia-clearing in nephrotic rats. *Ibid*, **36**, 700-705.

——, ——, and Byers, S. O. (1956). The causal role of plasma albumin deficiency in experimental nephrotic hyperlipemia and hypercholesteremia. *Ibid.*, **35**, 522-532.

Spitzer, J. A., and Spitzer, J. J. (1956). Effect of liver on lipolysis by normal and postheparin sera in the rat. *Amer. J. Physiol.*, **185**, 18-22.

Spitzer, J. J., and Roheim, P. S. (1958). Uptake and release of plasma unesterified fatty acids in the dog. *Fed. Proc.*, **17**, 155.

Stormont, J. M., and Mackie, J. E. (1959). An abnormality of non-esterified fatty acid metabolism in alcoholic patients with cirrhosis. *J. clin. Invest.*, **38**, 1047.

Wajchenberg, B. L., Hoxter, G., Mello, E. H. L., and Ulhoa Cintra, A. B. (1960). Non-esterified and esterified fatty acids in hepatocellular disease. *Lancet*, **1**, 1218-1219.

Gut, 1961, **2**, 240

The effect of vagotomy and pyloroplasty on the maximal acid response to histamine

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SYNOPSIS Pyloroplasty alone does not significantly alter the maximal acid response to histamine but it is considerably reduced in those patients who have had a vagotomy.

The role of the vagus in gastric acid secretion has become increasingly complex. A direct stimulating effect on the parietal cells of the body of the stomach and the release of gastrin from the pyloric gland area are facts which are now well established; sensitization of the antrum to local stimuli for the release of gastrin, a less well established function (Maung Pe Thein and Schofield, 1959). A role for the vagus as part of a vago-vagal reflex has recently also been postulated in the acid response of the fundus to local distention (Grossman, 1960).

In addition to these aspects, it has been known for a long time now that the responsiveness of the parietal cell to a wide variety of secretory stimuli is dependent on intactness of vagal innervation. In a considerable number of studies it has been shown that vagotomy reduces the acid response to histamine both in the dog and in man. In the published studies to date the dose of histamine studied has usually been small, and vagotomy in man has almost invariably been performed along with a gastroenterostomy for drainage.

The present study was suggested by the need to evaluate a series of patients with ulcer disease treated by vagotomy and pyloroplasty. This allowed us to study the reactivity of the intact and vagally denervated stomach without the interference of a gastroenterostomy.

To define the responsiveness of the human parietal cells somewhat more accurately, we elected to measure the 'maximal acid output to histamine' using the augmented histamine test of Kay (1953). This investigator has shown that the gastric acid output to four times the standard dose of histamine acid phosphate (0·04 mg./kg. body weight) approximates the maximum response. Card and Marks (1950) have demonstrated that the maximum acid output is a function of the number of parietal cells, although it is clearly recognized that the maximal acid output in response to histamine does not represent the maximal acid output of which the stomach is capable.

METHOD

Twenty patients with duodenal ulcer who had been subjected to vagotomy and pyloroplasty were studied. The augmented histamine test according to the technique of Kay was performed before surgery, and again one to three weeks following surgery. Six patients were restudied three months after operation.

Minor modifications were made in the procedure. Pyribenzamine, 100 mg. intramuscularly was used initially as the antihistamine. However, because this produced mausea in some patients, chlortrimeton, 10-20 mg. intramuscularly, was used with better results. Bromphenol blue and phenol red were used as the indicators for titration of free and total acid respectively. All anticholinergic drugs were stopped at least 12 hours before the test. Several studies were performed using a double lumen tube instead of the Levine tube. With one lumen in the duodenum and one in the stomach, we attempted to minimize the amount of gastric secretion lost into the duodenum.

To test the completeness of vagotomy, an insulin test according to the technique of Hollander (1946) was performed post-operatively in 10 patients, seven of whom had had significant reductions, and three with little or no reduction in acid response. In several patients with small reductions, lack of cooperation prevented further testing.

The data were calculated in terms of miliequivalents of free acid and total acid produced during the half-hour period from 15 to 45 minutes after histamine. The volume of parietal and non-parietal component was also calculated, according to the formula of Thompson and Vane (1953), when only acid concentration and volume of secretion are known.

RESULTS

A REPRODUCIBILITY OF AUGMENTED HISTAMINE TEST

Pre-operatively the test was repeated twice in four

patients and three times in two patients. The agreement in free acid production was within 10% except in one patient (Table I). The agreement in total acid was less marked.

TABLE I

Patient No.	*Acid*	*First Test*	*Second Test*	*Third Test*[2]	*Average*
1	Free	12·7[1]	12·6		12·6
	Total	21·6	16·6		19·1
2	Free	9·2	7·0		8·1
	Total	19·6	8·8		14·2
12	Free	13·9	13·6		13·8
	Total	17·0	21·8		19·4
13	Free	12·5	13·0	12·6	12·7
	Total	21·8	16·0	14·9	17·6
14	Free	10·6	11·5		11·0
	Total	20·5	14·6		17·6
20	Free	13·3	13·7	13·4	13·5
	Total	17·6	18·8	16·7	17·7

[1]Values expressed as milliequivalents per 0·5 hour.
[2]Third test performed in tandem to second.

B PATIENTS WITH INCOMPLETE VAGOTOMY Although it was felt at the time of operation that vagotomy had been successfully performed in all patients, postoperatively three patients had a marked rise in acid concentration after the injection of 20u. of insulin intravenously. The results (Table II) indicate that the changes observed in the augmented histamine test were of small magnitude. Pyloroplasty and incomplete vagotomy did not affect the augmented histamine response substantially.

C PATIENTS WITH VAGOTOMY AND PYLOROPLASTY Seventeen patients are included in this group, although only seven had negative post-operative insulin tests, the proven incomplete vagotomy being reported in section B. One patient was discarded from the study. His pre-operative value of 1·8 mEq. of free acid was so low that it was felt to be due to technical errors. The results in the remaining 16 patients are presented in Table III. There was an average decrease of 7·8 mEq. of free acid produced (an average decrease of 69%). Total acid response decreased by 10·2 mEq. (an average decrease of 61%). If the seven patients in the series with proven complete vagotomy are considered separately, the decrease in free acid averaged 71% and total acid 74%.

D LATE RESULTS (THREE MONTHS) AFTER OPERATION The results in six patients studied three months after operation are given in Table IV. The average decrease in free acid was 12·2 mEq., an average reduction of

TABLE II

CHANGES IN AUGMENTED HISTAMINE TEST IN THREE PATIENTS WITH INCOMPLETE VAGOTOMY

Case No.	*Free Acid (mEq./0·5 hr.)*			*Total Acid (mEq./0·5 hr.)*		
	Pre-operatively	*Post-operatively*	*Change*	*Pre-operatively*	*Post-operatively*	*Change*
14	11·0	9·7	−1·3 (−12%)	17·6	12·1	−5·5 (−31%)
19	13·1	15·8	+2·7 (+21%)	16·4	19·2	+2·8 (+17%)
20	13·5	13·2	−0·3 (−2%)	17·7	16·2	−1·5 (−8%)
Average			+0·4 (+2·3%)			−1·4 (−7·3%)

TABLE III

CHANGE IN AUGMENTED HISTAMINE TEST IN PATIENTS WITH VAGOTOMY AND PYLOROPLASTY

Case No.	*Free Acid (mEq./0·5 hr.)*			*Total Acid (mEq./0·5 hr.)*			*Insulin Test*
	Pre-operative	*Post-operative*	*Change*	*Pre-operative*	*Post-operative*	*Change*	
1	12·6	2·3	−10·3 (−82%)	19·1	6·1	−15·0 (−68%)	Negative
2	8·1	0	−8·1 (−100%)	14·2	0	−14·2 (−100%)	
3	2·7	2·3	−0·4 (−15%)	3·5	5·1	+1·6 (+46%)	
4	7·6	7·0	−0·6 (−8%)	13·0	9·6	−3·4 (−26%)	Negative
6	4·9	0·1	−4·8 (−98%)	8·4	0·2	−8·2 (−97%)	Negative
7	3·8	1·4	−2·4 (−63%)	5·5	4·8	−0·7 (−14%)	
8	19·1	5·9	−13·2 (−69%)	26·0	7·9	−18·1 (−69%)	Negative
9	25·0	0	−25·0 (−100%)	32·2	0·1	−32·1 (−100%)	Negative
10	9·3	7·9	−1·4 (−14%)	12·3	9·7	−2·6 (−21%)	Negative
11	15·3	0·1	−15·2 (−99%)	21·3	0·8	−20·5 (−96%)	
12	13·8	4·8	−9·0 (−65%)	19·4	12·2	−7·2 (−37%)	
13	12·7	4·2	−8·5 (−67%)	17·6	5·5	−12·1 (−69%)	Negative
15	6·4	0·1	−6·3 (−98%)	10·8	0·4	−10·4 (−79%)	
16	9·1	0	−9·1 (−100%)	10·1	0·5	−9·6 (−95%)	
17	9·7	5·8	−3·9 (−40%)	12·3	7·2	−5·1 (−41%)	
18	5·4	0	−5·4 (−100%)	7·1	1·5	−5·6 (−79%)	
Average			−7·8 (−69%)			−10·2 (−61%)	

80%. Total acid decreased 15·3 mEq., an average reduction of 77%.

E RESULTS IN PATIENTS STUDIED WITH DOUBLE LUMEN TUBE In three patients with significant acid reduction (average 61% free and 68% total acid), gastric and duodenal contents were collected simultaneously. Two patients were studied both pre-operatively and post-operatively; one was studied post-operatively (Table V). The volume of secretion collected from the duodenum appeared to be small as compared with the gastric volume. The *p*H and concentrations of acid in this collection indicated that a substantial part of this volume was derived from extragastric sources. These data support the idea that the amount of gastric secretion lost into the duodenum after pyloroplasty, although not measured precisely, was not significant.

F PARIETAL AND NON-PARIETAL COMPONENT The volume of parietal and non-parietal component in each test was calculated according to the formula of

TABLE IV

LATE CHANGES (THREE MONTHS) IN AUGMENTED HISTAMINE TEST IN PATIENTS WITH VAGOTOMY AND PYLOROPLASTY

Case No.	*Free Acid (mEq./0·5 hr.)*			*Total Acid (mEq./0·5 hr.)*		
	Pre-operative	*Three Months Post-operative*	*Change*	*Pre-operative*	*Three Months Post-operative*	*Change*
1	12·6	2·5	−10·1 (−80%)	19·1	4·3	−14·8 (−78%)
4	7·6	2·2	−5·4 (−44%)	13·0	5·3	−7·7 (−59%)
6	4·9	0·8	−4·1 (−83%)	8·4	3·1	−5·3 (−63%)
8	19·1	2·5	−16·6 (−87%)	26·0	3·9	−22·1 (−85%)
9	25·0	2·5	−22·5 (−90%)	32·2	4·9	−27·3 (−85%)
11	15·3	0·8	−14·5 (−95%)	21·3	1·4	−19·9 (−93%)
Average			−12·2 (−80%)			−15·3 (−77%)

TABLE V

VOLUME RECOVERED FROM TESTS USING DOUBLE LUMEN TUBE

Patient No.	*Test*	*Gastric*				*Duodenal*			
		Volume (ml./0·5 hr.)	*pH*	*Acid Concentration (mEq./l.)*		*Volume (ml./0·5 hr.)*	*pH*	*Acid Concentration (mEq./l.)*	
				Free	*Total*			*Free*	*Total*
8	Pre-operative	173	1	110	150	21	1	50	110
	Post-operative	77	1	77	102	25	1·5	47	80
10	Post-operative	87	1	90	110	5	5	—	—
11	Pre-operative	133	1	115	160	19	7	—	—
	Post-operative	15	3	5	54	16	7	—	—

TABLE VI

CHANGES IN PARIETAL AND NON-PARIETAL COMPONENT AFTER VAGOTOMY AND PYLOROPLASTY

Patient No.	*Parietal Component (ml./0·5 hr.)*			*Non-parietal Component (ml./0·5 hr.)*		
	Pre-operative	*Post-operative*	*Three Months Post-operative*	*Pre-operative*	*Post-operative*	*Three Months Post-operative*
1	132	49	32·8	61	47·5	24·2
4	104	79	36·7	81	70·4	20·3
8	153	55·3	32·6	20	21·7	25·4
9	209	28·9	37·3	29	95·1	51·7
11	133	7·2	14·5	7·2	7·8	20·5
12	126	103		0·5	96	
13	115	38·8		17	14·2	
15	81·7	4·2		48·3	5·8	
18	49·1	17·9		22·9	31·1	
2	91·2	0		21·3	9	
3	25·1	36		12·9	15	
6	59·5	2·9	24·6	25·5	5·1	19·4
7	41·4	36·2		26·6	18·8	
10	104·3	65·7		76·7	21·3	
16	66·4	21		16·6	30	
17	91	55·4		49	32·6	
Average	98·8	37·6	29·8	32·2	32·6	26·9

Thompson and Vane (1953) derived from the work of Fisher and Hunt (1950). This formula assumes that the gastric aspirate is a mixture of two components (acid and non-acid) varying in proportion, but each having a fixed composition. In the 16 patients considered to have had a complete vagotomy and pyloroplasty, the parietal secretion decreased on the average from 98·8 ml./0·5 hr. to 37·6 ml./0·5 hr. (average = 62%) (Table VI). The non-parietal component was unchanged, averaging 32·2 ml./0·5 hr. before surgery and 32·6 ml./0·5 hr. after surgery. The three patients with incomplete vagotomy had an average reduction in parietal component of 28%. In the seven patients studied three months postoperatively, parietal cell component averaged 29·8 ml./0·5 hr.

DISCUSSION

That the response of the human stomach to food, alcohol, and small doses of histamine is variable from day to day is well known. In a study of effects produced by a surgical procedure, the augmented histamine test of Kay may improve this situation somewhat. Measuring free acid, Kay reported a 5% variation between tests in intact stomachs. In the present study we found the variation between preoperative tests done both in tandem and on separate days well within 10% in all but one patient. The reproducibility of the total acid response was not as satisfactory. In some studies using this test, however, the results have been expressed in terms of total acid, which is more variable, at least in our hands. Postoperatively, when the amounts of acid were small, the problem of collection was magnified, and small variations in total amount represented large percentage changes. Hence, the variability between tests was greater. When more than one post-operative study was done, the larger acid response was selected for calculation.

The results of the present study indicate that complete vagotomy reduced markedly the maximal acid response to histamine. This reduction was present on the first post-operative test as early as one to three weeks after vagotomy. When the test was repeated after three months, in most instances the reduction did not differ significantly from the early postoperative results.

Other investigators have been interested in the effect of vagotomy on responsiveness to histamine. Many of the earlier studies used submaximal doses of histamine and were on animals. Most recent studies have used patients with gastroenterostomy. Pyloroplasty is to be preferred because the parietal cell area is not incised. Pylorectomy, in which only a small longitudinal strip of tissue, straddling the pyloric sphincter, is removed to provide better drainage, is also more suitable, but adds the problem of removal of some pyloric gland area.

In 1946, three reports appeared on this problem in which patients undergoing supradiaphragmatic vagotomy were studied. Moore, Chapman, Schulz, and Jones (1946) found the response to histamine was unchanged. Thornton, Storer, and Dragstedt (1946), using the same procedure with a dosage of histamine dihydrochloride of 0·1 mg./kg. body weight, found similar results. However, in the third report, Grimson, Taylor, Trent, Wilson, and Hill (1946), using 0·4 mg. histamine, found that in general the response after vagotomy was lowered, but results were variable. Stein and Meyer (1948) also reported a decreased responsiveness. Using histamine phosphate (1 mg.), they found a reduction of 72% in patients with vagotomy alone.

Oberhelman and Dragstedt (1948) used isolated dog stomachs. At a dosage of 1 mg. of histamine phosphate, the total acid decreased 62, 77, and 60% in three dogs. They also found that atropine caused a marked decrease in histamine responsiveness both before and after vagotomy. This is supported by many studies, including the work of Janowitz and Hollander (1956), who showed that in dogs receiving large enough doses of atropine, histamine stimulation is virtually completely inhibited. Oberhelman and Dragstedt also found that in man with vagotomy and no drainage procedure, using histamine phosphate 1 mg., the response was markedly reduced.

Antia and Ivy (1949) and Antia, Rosiere, Robertson, and Grossman (1951), using dogs with gastric fistula, found marked reduction with histamine dihydrochloride (0·1 mg./kg.) (average = 57%). Using histamine base (0·06 mg./kg.), the reduction ranged from 24 to 84%. In those animals studied again one year later, the reduction was 82%. They put forward the suggestion that the decreased responsiveness was due to a decrease in production of acetylcholine with loss of sensitizing or potentiating action.

Code (1953), however, could not demonstrate the reduced responsiveness to vagotomy. Using standard and maximum histamine response in dogs with both innervated and denervated pouches, he found vagotomy left 'more or less unchanged the response to histamine'.

Farmer, Howe, Porell, and Smithwick (1951) demonstrated a reduction of 42% using histamine acid phosphate (0·4 mg.) in man after vagotomy and posterior gastroenterostomy. In comparing the response to a variety of stimuli in patients undergoing either oesophagectomy, antral exclusion, both with and without vagotomy, Waddell (1958) also found a decrease in response to histamine; the dosage unfortunately was not mentioned. More

recently, using the augmented histamine test, in patients undergoing vagotomy and gastroenterostomy, Falconer (1959) reported an average reduction of 70·8%, and Gillespie, Clark, Kay, and Tankel (1960), with the same procedure, an average reduction of 79·3%. Our findings of a reduction in free acid of 69·3% and in total acid of 60·7% is consistent with the results of most investigators.

In our study results were also calculated in terms of parietal and non-parietal secretion. This method of calculation derives from the work of Fisher and Hunt (1950). Formulae have been derived on the assumption that gastric juice is of two components. In carefully performed experiments where contamination via oesophagus and duodenum was kept to a minimum, calculated values approximated very closely the observed data. Making the further assumption that both components are produced with a constant fixed composition, Thompson and Vane (1953) derived a formula requiring knowledge of only volume of secretion and concentration of acid. Using this formula, we found that the parietal component was reduced following vagotomy from 98·8 ml./0·5 hr. to 37·6 ml./0·5 hr., a reduction of 62%. Non-parietal component, which was 32·2 ml./0·5 hr. before operation, averaged 32·6 ml./0·5 hr. post-operatively. These results indicate that if histamine has any effect on the production of non-parietal component, it is not altered by vagotomy.

If at the dosage of histamine used in the augmented histamine test, all parietal cells capable of secreting acid are functioning close to the maximum, then individual variation should be related to variation in parietal cell mass. Card and Marks' study, in which they performed cell counts on tissue removed at subtotal gastrectomy and correlated this with changes in response to the augmented histamine test, indicates that this is indeed so. However, it has been shown that without altering the stomach, the response to the augmented histamine test may be changed. Gregory (1958) reported an increased response after portal vein ligation, and Clarke, Neill, and Welbourn (1960) produced an increase by prolonged administration of steroids. Clarke found an increase in the number of parietal cells in this latter study.

In the present study, it is unknown whether the decrease is due to an atrophy of some parietal cells or to a generalized reduction in secretory capacity. The finding of reduction as early as one to three weeks post-operatively suggests the latter, but is by no means conclusive. Whether this reduction is due to an effect directly on the parietal cell or possibly to an alteration in the vascular supply of the tissue is also not known. While the isolated mucosa is capable of responding to histamine *in vitro*, Thompson and Vane (1953) have demonstrated that the secretory response to histamine may be correlated with blood flow in the stomach.

Other than the theoretical importance of the problem investigated in this study, there is significant clinical importance. If the ability of the stomach to respond to stimulation is so markedly reduced, the operation of vagotomy and pyloroplasty seems to offer promise. Two disturbing features, however, are seen in this study. One is that three patients out of 10 had positive insulin tests, and their responses to the augmented histamine test were unchanged from pre-operative values. The other is that not all patients had large reductions, some being much less than average.

SUMMARY

Twenty patients who underwent vagotomy with pyloroplasty were studied by the augmented histamine test pre-operatively and post-operatively. Six were studied again three months after surgery.

Pyloroplasty alone did not significantly alter the response to the augmented histamine test.

In 16 patients with vagotomy, free acid was reduced 7·8 mEq./0·5 hr., a decrease of 69%. The total acid was reduced 10·2 mEq./0·5 hr., a decrease of 61%. After three months, free acid was reduced 12·2 mEq./0·5 hr., an 80% decrease. Total acid was reduced 15·3 mEq./0·5 hr., a 77% decrease.

The parietal component was reduced from 98·8 ml. to 37·6 ml. in the post-operative period and to 29·8 ml. after three months. The non-parietal component was virtually unchanged. Some of the theoretical and clinical implications of the marked reduction in response to the augmented histamine test are discussed.

ADDENDUM

Since the submission of this report, six patients were studied again one year or more after operation. In one of these, in whom an insulin test was positive post-operatively, there was no significant change from the pre-operative values. In the five other patients, there was an average decrease in free acid of 8·4 mEq. (an average reduction of 83%). Total acid was decreased on the average 11·8 mEq. (an average reduction of 76%).

REFERENCES

Antia, F., and Ivy, A. C. (1949). Effect of vagotomy on gastric secretion and emptying time in the dog. *Fed. Proc.*, **8**, 5.

——, Rosiere, C. E., Robertson, C., and Grossman, M. I. (1951). Effect of vagotomy on gastric secretion and emptying time in dogs. *Amer. J. Physiol.*, **166**, 470-479.

Card, W. I., and Marks, I. N. (1950). The relationship between the acid output of the stomach following 'maximal' histamine stimulation and the parietal cell mass. *Clin. Sci.*, **19**, 147-163.

Clarke, S. D., Neill, D. W., and Welbourn, R. B. (1960). The effects of corticotrophin and corticoids on secretion from denervated gastric pouches in dogs. *Gut.*, **1**, 36-43.

Code, C. F. (1953). Do the vagus nerves have an inhibitory influence on gastric secretion? *Fed. Proc.*, **12**, 26.

Falconer, C. W. A. (1959). Discussion on the surgical management of chronic duodenal ulcer. *Proc. roy. Soc. Med.*, **52**, 840-842.

Farmer, D. A., Howe, C. W., Porell, W. J., and Smithwick, R. H. (1951). The effect of various surgical procedures upon the acidity of the gastric contents of ulcer patients. *Ann. Surg.*, **134**, 319-331.

Fisher, R. B., and Hunt, J. N. (1950). The inorganic components of gastric secretion. *J. Physiol.* (*Lond.*), **111**, 138-149.

Gillespie, I. E., Clark, D. H., Kay, A. W., and Tankel, H. I. (1960). Effect of antrectomy, vagotomy and gastrojejunostomy, and antrectomy with vagotomy on the spontaneous and maximal gastric acid output in man. *Gastroenterology*, **38**, 361-367.

Gregory, R. A. (1958). Gastric secretory responses after portal venous ligation. *J. Physiol.* (*Lond.*), **144**, 123-137.

Grimson, K. S., Taylor, H. M., Trent, J. C., Wilson, D. A., and Hill, H. C. (1946). The effect of transthoracic vagotomy upon the functions of the stomach and upon the early clinical course of patients with peptic ulcer. *Sth. med. J.* (*Bgham, Ala.*), **39**, 460-472.

Grossman, M. I. (1960). Stimulation of acid secretion by distention of the fundic part of the stomach (abstract). *Physiologist*, **3**, 68.

Hollander, F. (1946). The insulin test for the presence of intact nerve fibers after vagal operations for peptic ulcer. *Gastroenterology*, **7**, 607-614.

Janowitz, H. D., and Hollander, F. (1956). Effects of atropine on histamine-stimulated gastric secretion in the dog. *Amer. J. Physiol.*, **186**, 373-376.

Kay, A. W. (1953). Effect of large doses of histamine on gastric secretion of HCl. *Brit. med. J.*, **2**, 77-80.

Maung Pe Thein, and Schofield, B. (1959). Release of gastrin from the pyloric antrum following vagal stimulation by sham feeding in dogs. *J. Physiol.* (*Lond.*), **148**, 291-305.

Moore, F. D., Chapman, W. P., Schulz, M. D., and Jones, C. M. (1946). Transdiaphragmatic resection of the vagus nerves for peptic ulcer. *New Engl. J. Med.*, **234**, 241-251.

Oberhelman, H. A., Jr., and Dragstedt, L. R. (1948). Effect of vagotomy on gastric secretory response to histamine. *Proc. Soc. exp. Biol.* (*N.Y.*), **67**, 336-339.

Stein, I. F., Jr., and Meyer, K. A. (1948). Studies on vagotomy in the treatment of peptic ulcer. *Surg. Gynec. Obstet.*, **87**, 188-196.

Thompson, J. E., and Vane, J. R. (1953). Gastric secretion induced by histamine and its relationship to the rate of blood flow. *J. Physiol.* (*Lond.*), **121**, 433-444.

Thornton, T. F., Jr., Storer, E. H., and Dragstedt, L. R. (1946). Supradiaphragmatic section of the vagus nerves. *J. Amer. med. Ass.*, **130**, 764-771.

Waddell, W. R. (1958). Secretory function of the stomach after denervating operations. *J. appl. Physiol.*, **12**, 468-472.

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The measurement of intestinal sounds in man and their relationship to serum 5-hydroxytryptamine

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SYNOPSIS Carbachol and magnesium sulphate were used to increase the motility of the intestine in healthy volunteers. The effect was estimated by recording changes in abdominal sounds. Serum 5-hydroxytryptamine levels were assayed and found to be higher after gastrointestinal stimulation.

5-Hydroxytryptamine (5-HT) is probably produced in the argentaffin cells in the intestine. Before the identification of the substance enteramine with 5-HT, Erspamer had shown its presence in extracts of the alimentary tract, and Feldberg and Toh (1953) found that the distribution of 5-HT corresponded to that of the argentaffin cells.

A crucial experiment to demonstrate the origin of 5-HT was performed by Toh (1954). Using the technique of stomach perfusion devised by Dale and Feldberg (1934), he showed that there was a higher concentration of 5-HT in the portal blood of dogs than in the blood at the periphery. Similar experiments have recently been performed in patients by means of a cardiac catheter (Erspamer and Testini, 1959). They demonstrated more 5-HT in the hepatic vein than in the blood of the inferior vena cava. Haverback and Davidson (1958) reported extremely low levels of blood 5-HT in a patient with extensive resection of the large and small bowel, and Rosenberg, Davis, Moran, and Zimmermann (1959) showed that resection of the gastrointestinal tract in dogs reduced the serum 5-HT. The half-life of radioactive 5-HT in the bowel is shorter than in the platelets (Udenfriend and Weissbach, 1958), suggesting that platelets obtain 5-HT from the alimentary tract.

It may be that 5-HT has a function as a local hormone. Diarrhoea and excessive borborygmi have long been noted as a symptom of carcinoidosis in which excess 5-HT is produced (Waldenström and Ljungberg, 1955). Lembeck (1958), in a review of published cases, points out that the symptom of diarrhoea was mentioned by 43 of 51 patients who were specifically asked. Injection of up to 3 mg. of 5-HT intravenously in man produced increased intestinal motility in 80% of their subjects, according to Hendrix, Atkinson, Clifton, and Ingelfinger (1957), who recorded the contractions of the upper jejunum with balloons. Likewise Haverback, Hogben, Moran, and Terry (1957) recorded increased jejunal motility in anaesthetized dogs given 0·01 mg./kg./min. intravenously. Using guinea-pig ileum Bülbring and Crema (1958) studied the action of 5-HT on peristalsis. They found that in all concentrations 5-HT increased the effect of acetylcholine in contracting the ileum; when applied to the serosal surface it stimulated peristalsis but when applied to the mucosal surface it first sensitized then desensitized the preparation. When the intraluminal pressure was controlled, Bülbring and Crema (1959) were able to show that increasing the pressure caused liberation of 5-HT. In the intact anaesthetized animal they showed that increased pressure produced peristalsis as well as releasing 5-HT. They postulated that the enterochromaffin cells found frequently along the base of the epithelium might respond to a deformation due to pressure by release of 5-HT. In patients with diarrhoea due to non-tropical sprue, Kowlessar, Williams, Law, and Sleisenger (1958) demonstrated an increased urinary 5-hydroxyindoleacetic acid excretion, but Connell, Rowlands, and Wilcox (1960) could find no significant increase on repeating this experiment. It is tempting to postulate, therefore, that distension of the lumen of the gut causes 5-HT to be secreted by the enterochromaffin cells and that this results in increased peristalsis. In patients with carcinoid tumours, a flush comparable to that produced by intravenous 5-HT can be precipitated by a variety of stimuli. Thorson (1956) lists emotion,

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spices, and food; Snow, Lennard-Jones, Curzon, and Stacey (1955) record alcohol as a precipitant, and Bleehen (1955) reported a patient in whom a fatty meal caused flushing. Thorson (1956) produced a flush by giving a small meal after a fast, and Daugherty, Manger, Roth, Flock, Childs, and Waugh (1955) produced flushing by palpation of the tumour and by injection of histamine and adrenalin. Little is known about the effect of these stimuli on the liberation of 5-HT in the normal subject and its precise role in the alimentary tract is still not established. It may be that it is released during increased intraluminal pressure, and therefore if gastrointestinal activity could be increased artificially, an increased secretion of 5-HT might be detected. This possibility has been investigated in the experiments to be described.

METHODS

Volunteers were obtained from medical students and members of the staff of this college. Venepuncture was performed and clotting and release of 5-HT were prevented by withdrawing 16-20 ml. of blood into siliconed syringes (Holzbauer and Vogt, 1954; Stacey, 1958). The blood was withdrawn smoothly and without bubbling so as to ensure minimal liberation of 5-HT at this time (Stacey, 1958). The blood was rapidly transferred to two non-siliconed Pyrex centrifuge tubes, maintained at a constant temperature of 37°C. in a water-bath. Clotting was allowed to take place at 37°C. for 30 minutes. Vigorous stirring was avoided, as Sharman and Sullivan (1956) found that agitation of the blood sample during clotting reduced the 5-HT level. Various authors have adopted different periods of incubation for releasing the 5-HT. Sharman and Sullivan incubated the blood for 30 minutes at 37°C.; Zucker and Borrelli (1955) stated that there was no difference in the quantity of 5-HT measured after incubation for different lengths of time, but gave no figures in support of their claim. Pare, Sandler, and Stacey (1957) incubated the blood for 60 minutes. The important consideration, however, is to keep the conditions standard throughout the experimental work, and there was no significant difference between the levels of 5-HT measured after 30 minutes' or after 60 minutes' incubation at 37°C. (Adams, 1960). After 30 minutes' incubation, the tubes were transferred to a centrifuge and spun at 1,800 *g* for 30 minutes at room temperature. By this means, the clot and red cells are both spun to the bottom leaving supernatant serum. One millilitre of serum was added to 19 ml. of acetone in a stoppered centrifuge tube, then stored overnight in a refrigerator at 6°C. The solution was filtered to remove the protein and substances insoluble in 95% acetone. The solute was collected and placed in an incubator at 37°C., and the acetone removed *in vacuo*. This method is essentially that used by Hardisty and Stacey (1955). The residuum was then made up to 20 or 25 ml. with modified De Jalon's fluid, consisting of Na, 160 mEq./l.; K, 6·2 mEq./l.; Ca, 1·1 mEq./l.; Cl, 162·4 mEq./l.; HCO_3, 6·2 mEq./l.; dextrose, 2·8 mEq./l.; and hyoscine hydrobromide, 2 μg./ml.

METHOD OF ASSAY

The assays were all performed on superfused uteri of virgin rats, in oestrus induced by injecting 250 μg. stilboestrol in arachis oil intraperitoneally 18-24 hours before the assay. They were then killed and the uterus removed and placed in an organ bath surrounded by a water-jacket kept at 37°C. The saline and drug solutions were delivered by a dropper on to the uterus. Throughout the work a Casella automatic assay machine was used with minor modifications necessary for superfusion. A 4 × 4 Latin square design was used for each assay and the automatic assay machine was set up to give the doses in this order.

A dose response curve was constructed before each group of assays to enable the selected doses for the assay to be on the steep part of the curve. Two dilutions of standard 5-HT were used, usually in the ratio of two to one. The dilutions of the unknown were used in approximately the same strengths, and also in the same ratio to one another as the standards. In this way the calculation used by Schild (1942) could be applied to the results. The results are given in terms of 5-hydroxytryptamine base.

MEASUREMENT OF GASTROINTESTINAL ACTIVITY

A microphone (Philips No. EL3750/00) having a diameter of 7 cm. was embedded in a ring of sponge rubber. This was surrounded by a ring of cork which rested in a polymethyl meth acrylate box 19 cm. × 19 cm. × 10 cm. The space between the microphone and box was padded with cotton wool. The microphone, so supported, was placed on the subject's abdomen and the box was held in position with a leather strap. This technique was found to be efficient for excluding extraneous noise. The microphone lead was connected to a tape recorder (Philips type AG 81085) which allowed three speeds of tape. The recording was made with maximum input amplification at a tape speed of 4·75 cm./sec. For short experiments the microphone was kept in position throughout; for experiments lasting longer than three hours, the microphone was removed after the initial recording and later replaced in the same position. Later the information stored on the magnetic tape was converted to electrical pulses which were amplified to approximately 70 volts by an R/C coupled amplifier and passed through a discriminator built into a scaling unit. The discriminator accepted only the electrical pulses which were larger than 70 volts and which had a rapidly rising wave front. These pulses were further amplified and then counted on three Mullard stepping tubes (Z303C) and a mechanical register. The amplifier and filter were arranged so that only loud intestinal noise was in a correct form to be accepted by the stepping tubes and counter. The steps in this amplification and filtering were initially followed on an oscilloscope and photographed. The tape recording was played back at four times the recording speed (19 cm./sec.). The number of counts per minute playing time was recorded, as well as the total number of counts over the duration of the experiment. Two measurements were therefore obtained: one recording the number of counts for each four-minute period, the other giving the average count

per minute for the duration of the recording. A graph could then be plotted showing the variation of the counts with time before and after a stimulus, and also the average level of counts before and after the stimulus.

One group of subjects received 25 g. of magnesium sulphate dissolved in 250 ml. of water by mouth. Another group received 0·20 mg. carbachol subcutaneously. The first sample of blood was withdrawn immediately before the injection or ingestion of the drug. The second blood sample was withdrawn one hour after the carbachol injection and four hours after the ingestion of magnesium sulphate.

The addition of brom lysergic acid diethylamide, 1 μg./ml., to the solution of drug and the extract of serum caused inhibition of the response of the uterus to both standards and unknown. This indicates that the substance extracted from the serum is 5-HT (Gaddum, 1958).

RESULTS

In order to ensure that repeated venepuncture or any alteration in physical activity did not produce a systematic and significant effect upon the levels of 5-HT measured, the following exploratory studies were performed. Five subjects were made to exercise at 350 kg./m. per minute for 10 minutes by means of a step test (Hugh-Jones and Lambert, 1952). Three samples of blood were obtained from each volunteer; one before exercise, one immediately after exercise, and one 30 minutes later. The results (Table I) show

TABLE I

EFFECT OF EXERCISE AND REPEATED VENEPUNCTURE ON SERUM 5-HT

Subject	*Serum 5-HT (μg./l.)*		
	Before Exercise	*Immediately after Exercise*	*30 min. after Exercise*
1	223	323	217
2	91	109	72
3	279	178	184
4	111	121	111
5	146	149	151

no significant difference between 5-HT levels measured at these times (P = >20%). It may be concluded that repeated venepuncture has no effect upon the levels of 5-HT nor has variation in the amount of physical exercise performed by the subjects.

Four other subjects were given injections of saline subcutaneously and the gastrointestinal activity was measured before and after this injection. No significant difference between the levels could be found. (Mean pre-injection noise level, 6·5 sounds/min.; mean post-injection noise level 7·0 sounds/min.). This therefore shows that an injection of an inert substance does not alter the level of gastrointestinal noise as measured in this way.

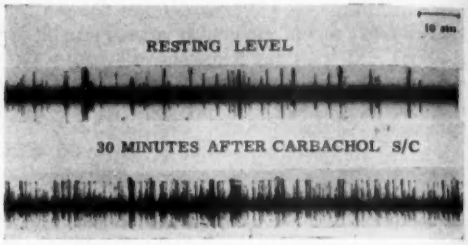

FIG. 1. *The effect of* 0·2 *mg. carbachol on gastrointestinal noise recorded on electrical tape.*

THE EFFECT OF CARBACHOL Gastrointestinal noise was recorded before and after the subcutaneous injection of 0·2 mg. carbachol into five subjects. The signals on the tape were counted as outlined above. Figure 1 shows a photograph of the oscilloscope recording of the tape before and after an injection of carbachol; changes in the counts per minute are shown graphically in Figure 2. The standard deviation of the counts made before injecting the drug was calculated. This standard deviation multiplied by 3 indicates the upper level of recording that would be exceeded only 1·5 in a thousand times supposing the drug had no effect. This is a convenient arbitrary boundary which has been used to determine the time of onset of drug action. The hatched line across the graphs indicates this level. The mean levels before and after injection are shown in Table II.

TABLE II

EFFECT OF INJECTIONS OF CARBACHOL ON GASTROINTESTINAL NOISE

Subject	*Abdominal Sounds per Minute (mean ± S.D.)*	
	Before Drug	*After Drug*
1	13·3 ± 5·5	63·3 ± 8·4
2	1·0 ± 1·2	46·0 ± 10·2
3	2·5 ± 0·6	27·5 ± 5·9
4	4·5 ± 2·9	20·9 ± 3·6
5	10·0 ± 4·1	131·0 ± 23·4

It is evident that subcutaneous injection of carbachol increased the amount of gastrointestinal noise. The subjects reported various symptoms; they were occasionally aware of increased bowel movement, alteration in accommodation of the lens, and increased salivation. Blood was withdrawn immediately before and one hour after injection of carbachol. Assays of the 5-HT level of this blood were performed and are tabulated in Table III, which shows that there was a consistent rise in 5-HT content of the second sample (P < 0·05). It is evident that injection of carbachol increases both gastrointestinal noise and serum 5-HT.

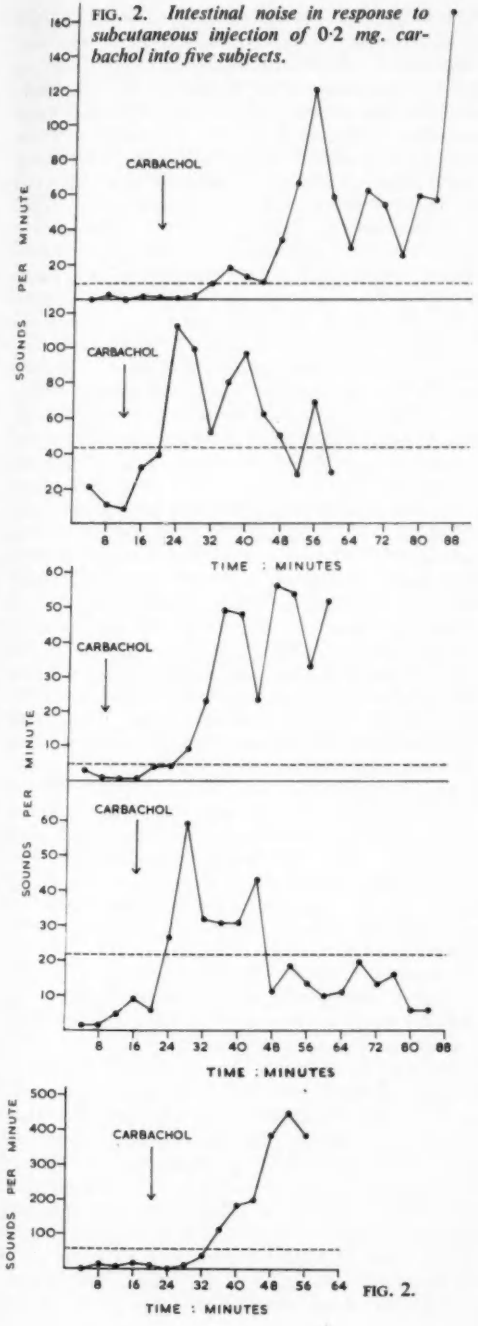

FIG. 2. *Intestinal noise in response to subcutaneous injection of 0·2 mg. carbachol into five subjects.*

TABLE III

EFFECT OF INJECTIONS OF CARBACHOL ON SERUM 5-HT

Subject	*Serum 5-HT (μg./l.)*	
	Before Drug	*After Drug*
1	139	177
2	134	151
3	138	171
4	139	183
5	144	227

EFFECT OF MAGNESIUM SULPHATE In a further five subjects 25 g. of magnesium sulphate was given dissolved in 250 ml. of water. Gastrointestinal noise was recorded before and three hours after the ingestion of this drug, and it can be seen from Table IV that

TABLE IV

EFFECT OF INGESTION OF MAGNESIUM SULPHATE ON GASTROINTESTINAL NOISE

Subject	*Abdominal Sounds per Minute (mean ± S.D.)*	
	Before Drug	*After Drug*
6	5·7 ± 0·7	56·1 ± 12·7
7	2·8 ± 0·8	117 ± 16·1
8	7·8 ± 1·9	11·2 ± 1·1
9	2·0 ± 0·7	2·7 ± 1·0
10	3·3 ± 1·1	24·8 ± 6·2

gastrointestinal noise was increased. In a similar way to the experiments using carbachol samples of blood were taken before the ingestion of magnesium sulphate and four hours afterwards. It will be seen from the results (Table V) that in four out of five

TABLE V

EFFECT OF INGESTION OF MAGNESIUM SULPHATE ON SERUM 5-HT

Subject	*Serum 5-HT (μg./l.)*	
	Before Drug	*After Drug*
6	158	177
7	110	149
8	253	268
9	226	226
10	173	196

experiments the serum 5-HT was increased after the ingestion of magnesium sulphate. An analysis of variance confirms that the increase in serum 5-HT is significant ($P < 0.05$). Combining the results from the two groups of experiments gives a positive correlation between 5-HT increase and the increase in gastrointestinal noise ($r = +0.726$ which is significant $0.02 > P > 0.01$). It would appear therefore that an increase in gastrointestinal noise is correlated with increase in the serum 5-HT.

DISCUSSION

The problem of the measurement of gastrointestinal activity can be approached in two ways (Vaughan-Williams, 1954), either by using isolated preparations, or by recording motility in the whole animal. As man was to be the experimental subject, the latter approach was more convenient. Studies of the motility of the bowel can be made radiologically. However, it is difficult to obtain significant pictures and large numbers of films may have to be taken before an analysis can be attempted (McLaren, Ardran, and Sutcliffe, 1950). Such techniques involve personal observation only, with little if any measurement possible, and visualization is possible only for short periods (Code, Hightower, and Morlock, 1952). Balloon studies are simple and inexpensive but are also open to many objections (Code *et al.*). The technique is relatively unpleasant for the subject and the initial nausea may influence motility (Ingelfinger and Moss, 1942). Gruber and DeNote (1935) studied the effects of intraluminal balloons on recorded contractions. They found that increasing the size of the balloon caused an increased rate of contractions of the small intestine; increasing the pressure caused increased contraction and peristalsis, and if the balloon was too large contraction and relaxation of neighbouring intestinal segments caused fallacious readings. The main disadvantage of the balloon recordings for the present work was the fact that they only measure the change in one localized segment of the intestine. For the present purpose recording the sounds produced by the intestine appeared more convenient. In 1905, Cannon recorded intestinal noise graphically by connecting a microphone placed on the abdomen to an induction coil which when activated caused contraction of a nerve muscle preparation and this was recorded. He examined the sounds from different parts of the abdomen and concluded that rhythmical sounds emanating from different portions of the gut could be recorded. He described both the 'gurgling' noise which occurred when fluid and gas passed from a contracting segment to one which was relaxed, and the 'snapping' noise of gas bubbles. These two types of sound were labelled first and second degree bowel sounds by Du Plessis (1954) and were considered to be due to the same causes. Farrer and Ingelfinger (1955) devised a more satisfactory method for recording and measuring gastrointestinal noise. They recorded the noise on a magnetic tape and then played back the tape and followed the output on an oscilloscope. They also passed the electrical pulses through an integrator which therefore registered graphically the rate of change of the noise. Their analysis depends on the assumption that altered noise will reflect altered motility, provided the gas content of the bowel remains constant, and they maintain that there is no reason to suppose that the gas content would alter suddenly over the short period of measurement when experiments with drugs are used. They measured at the same time the motility by balloon and radiographs. There was often a discrepancy between the noise recorded and the motility as revealed by balloon studies. They consider that this was due to local stimulation of the intestine by the balloon. There was always a correlation between the two methods if drugs were used to produce widespread alteration in bowel movement. The authors concluded that the method had distinct advantages: it enabled recordings to be made with minimal interference with the subject; it enabled the results to be measured and treated quantitatively; and it may be more sensitive than other methods. Recording and measuring the noise produced by the intestine is not an ideal procedure because intestinal sound depends on many variables. The relative proportion of fluid and gas in the intestine influences the sound level; Milton (1958) demonstrated that an exteriorized loop of bowel completely filled with fluid was silent though still contracting. The thickness of the abdominal wall in different subjects will affect the amount of sound transmitted as will the position of the contracting bowel relative to the microphone. In these experiments the subject was used as his own control and therefore these variables are irrelevant. It is evident that in any one subject over the period of time required for the experiment the most important factor in alteration in sound level is the change in motility. In the present work a method similar to that of Farrer and Ingelfinger was used. It enabled a recording to be made of the sounds before and after administration of drugs. It is evident that the administration of both carbachol and magnesium sulphate increase the volume of noise recorded and it is, therefore, reasonable to assume that the motility of the bowel was increased likewise.

The present experiments show that this increase in motility was correlated with an increased release of 5-hydroxytryptamine into the blood.

Connell *et al.* (1960) measured the excretion of 5-hydroxyindoleacetic acid (5-HIAA) in the urine of patients with diarrhoea. They compared the levels with a control group; there was a slight increase but the difference was not significant. The level of 5-HIAA in the urine will depend more on the intake of 5-HT and tryptophan in the diet than on the small change in ratio between the 5-HT in the gut and in the platelets. The total amount of 5-HT in the gut is between 4·5 and 7·5 mg. (Erspamer and Testini, 1959). The mean increase in serum 5-HT in the

present experiments was 31 μg./l.; therefore the total quantity released into the circulation was approximately 100 μg. and this represents only 1·6% of the total 5-HT in the alimentary tract. This change may not be detectable in the urine, as in the experiments quoted above by Connell *et al.* an increased ingestion of approximately 6·0 mg. of 5-HT was required to produce an alteration in excretion of 5-HIAA which could be considered significant.

The present experiments throw no light upon the mechanism of activity of 5-hydroxytryptamine in the bowel but they do indicate that a similar state may occur in man to that demonstrated by Bülbring and Crema (1959) in guinea-pigs. In the intact animal there are several ways in which the 5-HT released from its site of storage or activity may be removed. First, it may diffuse in the lumen of the bowel, as in the experiments of Bülbring and Crema and also Leach (1960). Another way would be by the destruction by amine oxidase which has been found in the alimentary tract of man (Epps, 1945). A third possibility would be the diffusion into the smooth muscle cells and destruction there (Born, 1960). The fourth possibility is that the 5-HT would be removed by the platelets from its site of activity. This is quite likely to take place, as the blood capillaries form a rich network in the villi and are in close proximity to the basement membrane of the mucosa (Carleton and Short, 1954). Thus the platelets, with an avidity enabling them to take up 5-HT against a gradient of 1,000:1 (Born and Bricknell, 1959), come into very close contact with the site of liberation of the 5-HT from the enterochromaffin cells which lie, for the most part, along the basement membrane. It would be expected therefore that when an increased amount of 5-HT is liberated it would find its way into the platelets and be detected in the blood, and this, in fact, has occurred in the experiments outlined.

Stimulation of the bowel with 0·2 mg. carbachol subcutaneously produced an increase in gastrointestinal noise of a mean of 51 sounds/min. and the serum 5-HT was increased by a mean of 43 μg./l. Likewise with administration of magnesium sulphate a mean increase of intestinal noise of 38 sounds/min. occurred and a mean increase in serum 5-HT of 19 μg./l. There was a significant positive correlation between the change in intestinal noise and the increase in serum 5-HT. It seems, therefore, that the level of 5-HT in the serum depends at least partly upon the degree of gastrointestinal motility in man. This suggests that the platelets have a scavenging function and remove the 5-HT from its site of activity and that therefore the level in the blood does not represent the beginning of a physiological system but rather the end.

It is a great pleasure to acknowledge the help received from Mr. Denis Knight in constructing the recording apparatus. I also wish to record my thanks to Professor Weatherall for his interest and guidance during the progress of this work.

REFERENCES

Adams, B. G. (1960). Unpublished observations.
Bleehen, N. M. (1955). *Lancet*, **2**, 1362.
Born, G. V. R. (1960). Personal communication.
——, and Bricknell, J. (1959). *J. Physiol. (Lond.)*, **147**, 153.
Bülbring, E., and Crema, A. (1958). *Brit. J. Pharmacol.*, **13**, 444.
——, —— (1959). *J. Physiol. (Lond.)*, **146**, 18.
Cannon, W. B. (1905). *Amer. J. Physiol.*, **14**, 339.
Carleton, H. M., and Short, R. H. D. (1954). *Schafer's Essentials of Histology*, 16th ed. Longmans, Green, London.
Code, C. F., Hightower, N. C., Jr., and Morlock, C. G. (1952). *Amer. J. Med.*, **13**, 328.
Connell, A. M., Rowlands, E. N., and Wilcox, P. B. (1960). *Gut*, **1**, 44.
Dale, H. H., and Feldberg, W. (1934). *J. Physiol. (Lond.)*, **81**, 39*P*.
Daugherty, G. W., Manger, W. M., Roth, G. M., Flock, E. V., Childs, D. S., Jr., and Waugh, J. M. (1955). *Proc. Mayo Clin.*, **30**, 595.
Du Plessis, D. J. (1954). *S. Afr. med. J.*, **28**, 27.
Epps, H. M. R. (1945). *Biochem. J.*, **39**, 37.
Erspamer, V., and Testini, A. (1959). *J. Pharm. Pharmacol.*, **11**, 618.
Farrer, J. T., and Ingelfinger, F. J. (1955). *Gastroenterology*, **29**, 789.
Feldberg, W., and Toh, C. C. (1953). *J. Physiol. (Lond.)*, **119**, 352.
Gaddum, J. H. (1958). In *5-Hydroxytryptamine: Proceedings of a symposium held in London, 1957*, ed. Lewis, G. P. Pergamon Press, London.
Gruber, C. M., and DeNote, A. (1935). *Amer. J. Physiol.*, **111**, 564.
Hardisty, R. M., and Stacey, R. S. (1955). *J. Physiol. (Lond.)*, **130**, 711.
Haverback, B. J., and Davidson, J. D. (1958). *Gastroenterology*, **35**, 570.
——, Hogben, A. M., Moran, N. C., and Terry, L. L. (1957). *Ibid.*, **32**, 1058.
Hendrix, T. R., Atkinson, M., Clifton, J. A., and Ingelfinger, F. J. (1957). *Amer. J. Med.*, **23**, 886.
Holzbauer, M., and Vogt, M. (1954). *Brit. J. Pharmacol.*, **9**, 249.
Hugh-Jones, P., and Lambert, A. V. (1952). *Brit. med. J.*, **1**, 65.
Ingelfinger, F. J., and Moss, R. E. (1942). *Amer. J. Physiol.*, **136**, 561.
Kowlessar, O. D., Williams, R. C., Law, D. H., and Sleisenger, M. H. (1958). *New Engl. J. Med.*, **259**, 340.
Leach, G. D. H. (1960). Personal communication.
Lembeck, F. (1958). In *5-Hydroxytryptamine: Proceedings of a symposium held in London, 1957*, ed. Lewis, G. P. Pergamon Press, London.
McLaren, J. W., Ardran, G. M., and Sutcliffe, J. (1950). *J. Fac. Radiol.*, **2**, 148.
Milton, G. W. (1958). *Med. J. Aust.*, **2**, 490.
Pare, C. M. B., Sandler, M., and Stacey, R. S. (1957). *Lancet*, **1**, 551.
Rosenberg, J. C., Davis, R., Moran, W. H., and Zimmermann, B. (1959). *Fed. Proc.*, **18**, 503.
Schild, H. O. (1942). *J. Physiol. (Lond.)*, **101**, 115.
Sharman, D. F., and Sullivan, F. M. (1956). *Nature (Lond.)*, **177**, 332.
Snow, P. J. D., Lennard-Jones, J. E., Curzon, G., and Stacey, R. S. (1955). *Lancet*, **2**, 1004.
Stacey, R. S. (1958). In *5-Hydroxtryptamine: Proceedings of a symposium held in London, 1957*, ed. Lewis, G. P. Pergamon Press, London.
Thorson, A. H. (1956). *Amer. Heart J.*, **52**, 444.
Toh, C. C. (1954). *J. Physiol. (Lond.)*, **126**, 248.
Udenfriend, S., and Weissbach, H. (1958). *Proc. Soc. exp. Biol. (N.Y.)*, **97**, 748.
Vaughan-Williams, E. M. (1954). *Pharmacol. Rev.*, **6**, 159.
Waldenström, J., and Ljungberg, E. (1955). *Acta med. scand.*, **152**, 293.
Zucker, M. B., and Borrelli, J. (1955). *J. appl. Physiol.*, 7, 425.

Gut, 1961, **2**, 252

The cardiac sphincter in the cat

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SYNOPSIS A study of the function of the lower end of the oesophagus in the cat confirms that it behaves as a true physiological sphincter, even though histological evidence for such a structure is absent. Sphincteric action is a function of the circular muscle fibres in the lowest 2 cm. of the oesophagus. Reflex changes in tone of the sphincter are mediated through the autonomic nervous system.

The effects of possible neurohumoral transmitters, nerve stimulation, and antagonists have been studied.

In some laboratory animals (guinea-pig, rabbit, hamster, rat, mouse) a well-delineated anatomical sphincter at the cardia has been described, whereas in others (monkey, dog, and cat) the sphincter cannot be clearly defined by histological techniques (Botha, 1958a). Nevertheless, it is generally accepted that even though histological evidence is lacking, a sphincteric mechanism exists in these animals. Cannon (1908), Carlson, Boyd, and Pearcy (1922), and Knight (1934) all obtained physiological evidence for a sphincter in the cat, although different interpretations of the function, control, and exact location of the mechanism have been suggested. For instance, Nauta (1956) suggested that the sphincter acted only as a valve (in the dog) to prevent gastric reflux, and this interpretation has also been suggested for man (Barrett, 1954; Dornhorst, Harrison, and Pierce, 1954; Nauta, 1955, 1956), in whom the histological evidence for a sphincter is also lacking (Lendrum, 1937; Roux, 1939). On the other hand, Fyke, Code, and Schlegel (1956) and Atkinson, Edwards, Honour, and Rowlands (1957) regard this region in man as a true sphincter. Nor is there general agreement on the effects brought about by stimulation of the extrinsic nerve supply to the region, although they have been repeatedly studied (Langley, 1898; May, 1904; Klee, 1919; Koennecke, 1922; Veach, 1925, 1926). Some experiments have suggested that the response of the sphincter is dependent upon the coexisting gastric tonus (Carlson *et al.*, 1922; McCrea, McSwiney, and Stopford, 1925).

The present investigation defines the cardiac sphincter more accurately. The results of nerve stimulation and injected drugs suggest the mechanism of normal function. Opening of the cardiac sphincter can be induced either by increased oesophageal or gastric pressure. This effect is a function of the circular muscle fibres of the lower end of the oesophagus. Alterations in oesophageal and gastric pressure affect the sphincter both directly and reflexly.

METHODS

Male and female cats of 1·6 to 4·2 kg. were used, having been allowed water, but no food, for 12 hours before the experiment. After induction with ethylchloride and ether, five were spinalized by the method of Kosterlitz, Krayer, and Matallana (1954), two were decerebrated, and 34 were anaesthetized with chloralose (80 mg./kg.). Four animals were anaesthetized with sodium pentobarbitone (40 mg./kg.) by intraperitoneal injection.

In early experiments, a tube was passed through the pharynx into the oesophagus and a second tube was tied into a slit cut in the greater curvature of the stomach: these were each connected, via a reservoir, to separate water manometers. Either organ could be filled with saline and the pressure at which it emptied through the cardiac sphincter measured. The receiving organ, *i.e.*, oesophagus or stomach, was allowed to drain freely at atmospheric pressure during such an experiment.

The region of the gastro-oesophageal junction was also directly observed (*a*) by tying a wide-bore glass tube into a slit in the greater curvature of the stomach, with its distal end overlying the junction; (*b*) by using a cystoscope introduced in a similar way into the stomach; and (*c*) by observation of the oesophagus and stomach with the thorax and abdomen opened by a mid-line incision.

The lowest 2 cm. of the oesophagus was later established as the region with sphincteric action. In some experiments, as illustrated in Fig. 1, this region was cut free from muscular and mucosal connexion with the rest

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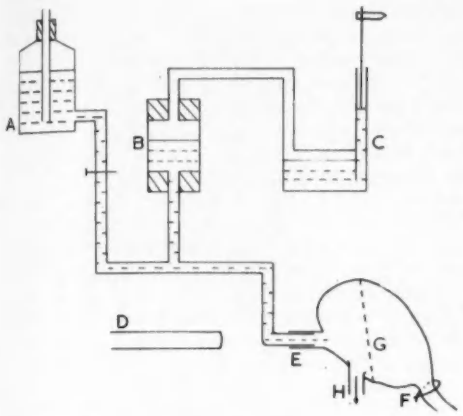

FIG. 1. *Saline from a Mariotte bottle (A) flows at constant pressure through a tube inserted into the lower end of the oesophagus (E) which is separated from the body of the oesophagus (D). The vagi remain intact and are stimulated in the neck. The stomach is occluded at the pylorus (F) but can be kept empty by the tube (H). A mucosal barrier (G) can be constructed so that the upper part of the stomach still remains empty and the remainder of the organ can be distended through another cannula tied in the greater curvature. Pressure changes in the lower oesophagus are transmitted to the reservoir (B) and recorded by water manometer (C) on a kymograph.*

of the oesophagus by a transverse incision, taking care to preserve the extramural blood vessels and extramural nerve fibres. The lumen of the isolated segment was cannulated and connected to a Mariotte bottle of saline kept at a height of 20 to 40 cm. above that of the oesophagus. A water manometer connected to a side arm measured the pressure at the point of inflow into the oesophageal segment. In this way the sphincter was used as a resistance to the free flow of saline (5-30 ml./min., usually 10 ml./min.) through the system and any change in tone was reflected as a change in the inflow pressure, contraction being registered as a rise in pressure. The saline drained into the stomach, which was kept empty by allowing free drainage through another cannula, to avoid pressure changes due to gastric contractions. The apparatus is shown in diagrammatic form in Fig. 1. The effect, on the isolated segment, of filling and emptying the main body of the oesophagus could also be studied in this preparation, as could the effects of nerve stimulation and injected drugs. By constructing a mucosal barrier (G) at the upper end of the stomach (Fig. 1) and inserting a second gastric cannula, the effect of distension of part of the stomach upon the tone of the sphincter was also investigated, without applying pressure directly to the sphincter itself.

The vagus nerves were stimulated in the neck using platinum electrodes, usually for periods of 30 sec. In order to stimulate the sympathetic supply the left gastric artery was divided and the distal end, along with the periarterial network of nerves, was pulled into a tubular polythene electrode holder in which two platinum rings spaced 0·5 mm. apart acted as electrodes. Supra-maximal square wave shocks (20 volts) of 2 msec. duration, and frequencies varying from 1/min. to 50/sec. were provided by a stimulator.

DRUGS Intravenous injections of the following drugs (doses expressed as base) were given through a cannula tied in a femoral or jugular vein: acetylcholine chloride, physostigmine sulphate, adrenaline hydrogen tartrate and noradrenaline hydrogen tartrate, histamine hydrogen phosphate, 5-hydroxytryptamine creatinine sulphate, isopropylnoradrenaline sulphate, nicotine hydrogen tartrate, hexamethonium bromide, hyoscine hydrobromide, and atropine sulphate.

RESULTS

As it traverses the diaphragm, the oesophagus is surrounded by the phrenico-oesophageal ligament, which further applies the extrinsic nerves to its external surface. The whole is enveloped in a layer of pleura above the peritoneum below the diaphragm, yet the relations of the diaphragm are variable. The lower end of the oesophagus is mobile, and while the stomach is not normally pulled into the thorax, the extent of the intra-abdominal part of the oesophagus varies considerably in different animals and in the same animal at different times.

If the passage of saline from a Mariotte bottle through the oesophagus is observed with a cystoscope tied into the stomach and focused on the gastro-oesophageal junction, then at low pressures rhythmic opening and closing movements are observed. If the pressure of the perfusing fluid is raised, these rhythmic movements are still observed, though the orifice never completely closes. The appearances are those of a rosette-shaped structure of which the movements recall those of pupillary hippus. A similar picture is observed when the saline is forced through the sphincter from the gastric side: as the stomach fills, the gastric mucosa slides away from the rosette, which finally opens and rhythmic movements start. These appearances are unaltered by dividing the diaphragm and dissecting the surrounding ligamentous structures, provided the blood supply is not greatly disturbed.

OPENING PRESSURES OF THE SPHINCTER The pressure at which fluid can be maintained without leakage in either the oesophagus or stomach gives a measure of the resistance to opening of the sphincter. If the oesophagus is filled, increasing the pressure in 2 ml. steps, the pressure rises until the sphincter opens, and the oesophagus empties, though not completely.

In spinal and decerebrate animals (seven experiments) this opening pressure was slightly higher (14-20 cm. H_2O) than in animals anaesthetized with chloralose (8-12 cm.). It was even lower (3-6 cm.) in four cats anaesthetized with nembutal. The sphincter closed again when the pressure had fallen to about half the opening pressure. This pattern, where a fairly high pressure was needed to open the sphincter, was observed in all but three animals (one spinal, two chloralose): in these, the opening pressure was only 2 cm. of H_2O. The opening pressure in response to direct oesophageal distension in all animals was unaffected by removal of the surrounding diaphragmatic musculature.

Under the alternative conditions, testing the resistance of the sphincter to increase in intragastric pressure, the results were more varied. The pressure at which the sphincter opened in response to increased gastric pressure was always higher than that needed when tested from the oesophageal side of the sphincter. It was usually greater in animals anaesthetized with chloralose (12-16 cm. H_2O) than in those anaesthetized with nembutal (6-13 cm. H_2O). Dividing the diaphragm did not alter these opening pressures materially except in two animals. In one chloralose cat the pressure required to force the sphincter fell from 18 to 11·5 cm. on dividing the diaphragm and in one nembutal animal it fell from 14·5 cm. to 7 cm.

It is obvious, therefore, that at the gastro-oesophageal junction there exists a mechanism which resists the passage of fluid in either direction and which may be called a sphincter. The sphincter is always more powerful in resisting gastric reflux than in preventing oesophageal emptying. Its resistance is higher in spinal animals than in those anaesthetized with chloralose and even lower if nembutal is used. Occasionally, the diaphragm appeared to augment the resistance of the sphincter to gastric reflux but did not affect oesophageal emptying.

EFFECTS OF VAGOTOMY ON OPENING PRESSURES With the oesophagus, sphincter, and stomach in continuity, bilateral vagotomy in the neck did not alter the opening pressure measured from the oesophageal aspect, but almost invariably produced an increased resistance to pressure exerted in the stomach. With the sphincter separated from the body of the oesophagus, vagotomy abolished the response to oesophageal or gastric pouch distension. The opening pressures of the isolated sphincter, however, were similar to those found with the gut in continuity. Again it was found that vagotomy raised the gastric opening pressure, leaving the oesophageal opening pressure unchanged.

DEFINING THE SITE OF THE SPHINCTER Electrical stimulation of the vagus (see later) causes contraction of the body of the oesophagus and relaxation of the cardiac sphincter. If a saline-filled, wide-bore (8 mm.) polythene tube is connected by a reservoir to a water manometer and has its tip situated in the mid-oesophagus it only records contractions of that part of the organ which is lower than the tip. A continuous flow of saline at about 10 ml./min. from a Mariotte bottle through the cannula results in a kymograph record of the alterations in pressure as the sphincter rhythmically contracts and relaxes. With the tip of the tube in this position vagal stimulation gives a contraction of the oesophagus which shows on the record as a sharp rise in pressure. As the tip of the tube is brought closer to the sphincter, a point is reached where stimulation results only in a pressure fall, due to relaxation of the sphincter. When the tube is advanced into the stomach, stimulation again results in contraction. Using the method where the sphincteric region is isolated from the other organs, these effects of vagal stimulation are even more strikingly demonstrated. Fig. 2 shows records of the pressure changes in the body of the oesophagus, the sphincteric segment, and the gastric pouch obtained simultaneously. The region where the response to stimulation is opposite to that of the body of the oesophagus and stomach is crudely defined as the lower 2 cm. of the oesophagus. This is also the region which can be seen to remain tightly contracted if either the stomach or the oesophagus is filled with saline, as in the experiments where the opening pressure was measured. The sphincter no longer resists opening after the circular muscle fibres have been divided by an incision down to the mucosa. This incision does not encroach upon the gastric musculature. It should also be noted that the sphincter becomes completely incompetent within a minute of death of the animal.

REFLEXES ASSOCIATED WITH DISTENSION So far, the results indicate that the cardiac sphincter is normally closed, but that an increase in pressure on either side of it leads to leakage of fluid through it. This opening of the sphincter may have been brought about by one or more of several mechanisms: a local or a central reflex leading to an active relaxation, a direct response of the muscle fibres to the pressure change, or even by a simple physical distortion of the sphincter.

The possibility of a central reflex was investigated using the sphincteric region isolated from the body of the oesophagus, continuously perfused through its lumen with saline. A mucosal barrier across the stomach also enabled the effects of gastric distension to be observed. Even though there was no direct

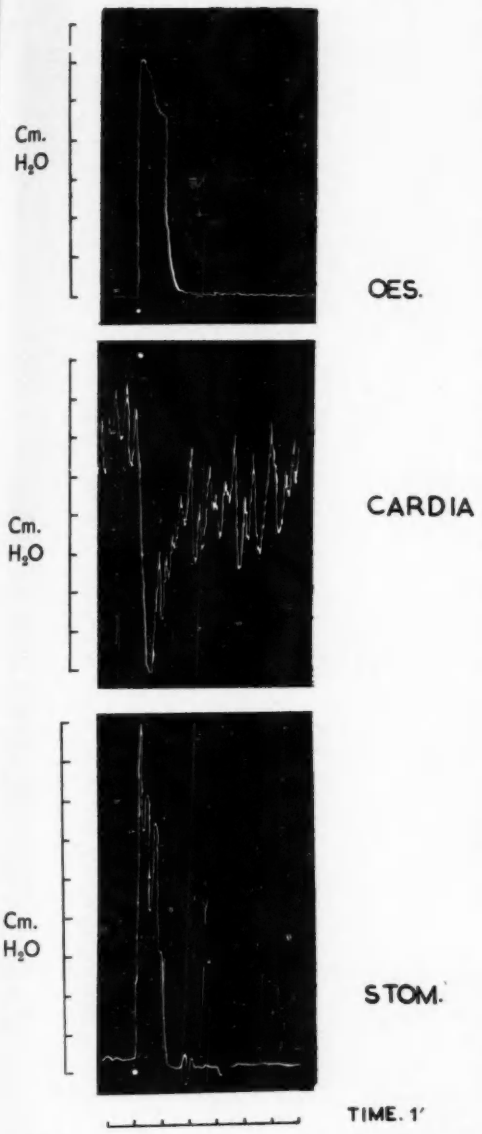

FIG. 2. *Cat, 3·4 kg. female, anaesthetized with chloralose. This record shows the effects obtained simultaneously from the body of the oesophagus, sphincter, and stomach pouch (isolated from the sphincter by a mucosal barrier) on stimulating the right cervical vagus nerve at 10 cycles per second for one minute. The centre record was obtained by perfusing the lumen of the sphincter with saline, as described in text. Time in minutes.*

muscular continuity, the cardiac sphincter relaxed when the oesophagus was distended with saline. The relaxation was roughly proportional to the amount of distension, was first seen in chloralose cats at an oesophageal pressure of 5 cm. of water, and was immediate in onset (Fig. 3). Emptying the oesophagus resulted in a brief increase in the tone of the sphincter (Fig. 3b, 3c); if the oesophageal distension was maintained, the sphincter gradually recovered its tone (Fig. 3d).

Distension of the gastric pouch gave effects which depended in a different way upon the amount of distension. With volumes up to about 40 ml. (leading to an increased gastric pressure of 4-6 cm. H_2O) the tone of the sphincter increased, but with larger volumes, the increase in tone was quickly followed by a relaxation which became more and more dominant with further distension of the gastric pouch. Some recovery of tone took place while the pouch remained distended, but this was less than in the similar circumstance noted with persistent oesophageal distension. On emptying the stomach, the sphincter became briefly hypertonic, and subsequently recovered its normal tone. These effects were diminished by unilateral and abolished by bilateral vagotomy, performed in the neck.

EFFECTS OF NERVE STIMULATION The effect of stimulating the vagus and sympathetic nerves was tested on the sphincter isolated from the oesophagus. If the central end of the right vagus, divided in the neck, was stimulated, the left nerve being intact, no appreciable response was obtained until the frequency was raised to 1/sec. At frequencies of 1/sec. to 50/sec., the sphincter relaxed, an effect which was delayed in onset at the lower frequencies. The degree of relaxation was maximal and the onset immediate at frequencies of 10 to 20 per sec. All these responses were abolished by cutting the contralateral vagus in the neck.

The peripheral end of the right vagus was stimulated at similar frequencies, and, again, only frequencies of 1/sec. to 50/sec. gave regular results. The character of the response obtained was not altered by the integrity of the other vagus. Relaxation of the sphincter, the normal response, was maximum at frequencies of 5 to 10 per sec. At higher frequencies, the response did not increase in magnitude but was sometimes more prolonged. At lower frequencies the response was smaller and there was often a delay in the onset of relaxation, or even a brief contraction preceding it (Fig. 4). Hexamethonium bromide in small doses (0·5 mg. intravenously) abolished both the early contractions and the delay, suggesting that it may be due to a balance between contractile and dilator elements, and in larger doses (10 mg./kg.)

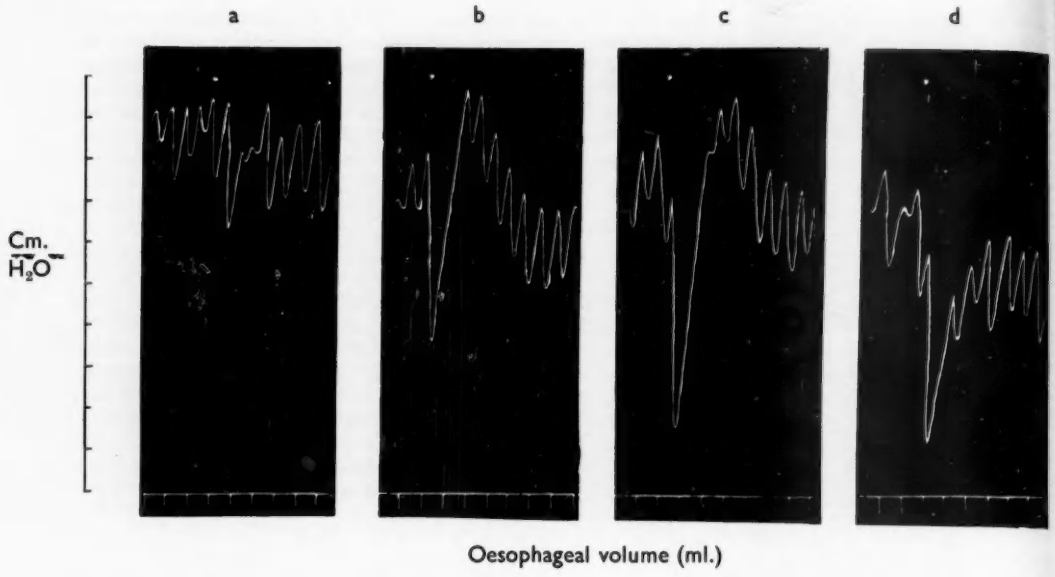

FIG. 3. *Cat, 3·2 kg. female, anaesthetized with chloralose. This records the effect obtained by the continuous flow method in the isolated sphincter of distending the body of the oesophagus. Only the vagus nerves connect the tissues. The oesophagus was filled rapidly and emptied after one minute (two minutes in d).*

The response obtained is relaxation of the sphincter, the degree depending on the volume of saline introduced. Relaxation is followed by an increase in tone of the sphincter when the oesophagus is emptied after one minute but this does not occur if emptying is delayed (d). Pressure changes in cm. H_2O, time scale one minute.

entirely abolished all responses. Atropine (0·5 mg. intravenously) also abolished first the delay preceding relaxation and eventually (2 mg./kg.) the entire response.

Division of one cervical vagus nerve had no effect on the intrinsic tone of the sphincter judged by the method of continuous flow, although bilateral vagotomy raised the tone, usually for two to three minutes, and occasionally for up to 30 minutes. However, when tested from the gastric side of the sphincter it was found that unilateral vagotomy raised the opening pressure from 12 to about 14 cm. H_2O and bilateral vagotomy raised it further to about 18 cm. H_2O (six out of seven animals.) This rise in tone persisted for the remainder of the experiment, a period varying up to three hours later.

The final path of the main sympathetic nerve supply to the cardiac sphincter is along the blood vessels to this structure. The major blood supply is from the oesophageal branch of the left gastric artery. Stimulation of these periarterial nerves entails division of the left gastric artery which produces an alteration in the rhythmic movements of the sphincter, slightly lowering the tone. It does not, however, abolish the resistance offered to perfusion. Stimulation of the sympathetic gave rise to substantial contractions of the sphincter, followed by an after-relaxation. This response was unaltered by hexamethonium (5 mg./kg.). The effect of atropine (2 mg./kg.) or hyoscine (2 mg./kg.) was to abolish the after-relaxation and to reduce the size of the contraction (Fig. 5).

EFFECT OF DRUGS ON THE CARDIA Acetylcholine (1-100 μg. intravenously) contracts the main body of the oesophagus and this is also its effect on the stomach. At the sphincter, relaxation always occurs, and a substantial effect is noted with doses of 5 to 10 μg. intravenously. The effect is potentiated by small doses of physostigmine (10-15 μg. intravenously), abolished by hyoscine (2-10 mg. intravenously), or atropine (2-10 mg. intravenously), and unaffected by hexamethonium (up to 25 mg. intravenously).

Adrenaline and noradrenaline (1-500 μg. intravenously) do not differ in their effects. An attempt was made to find if either drug was more active than the other but there was no apparent difference in the

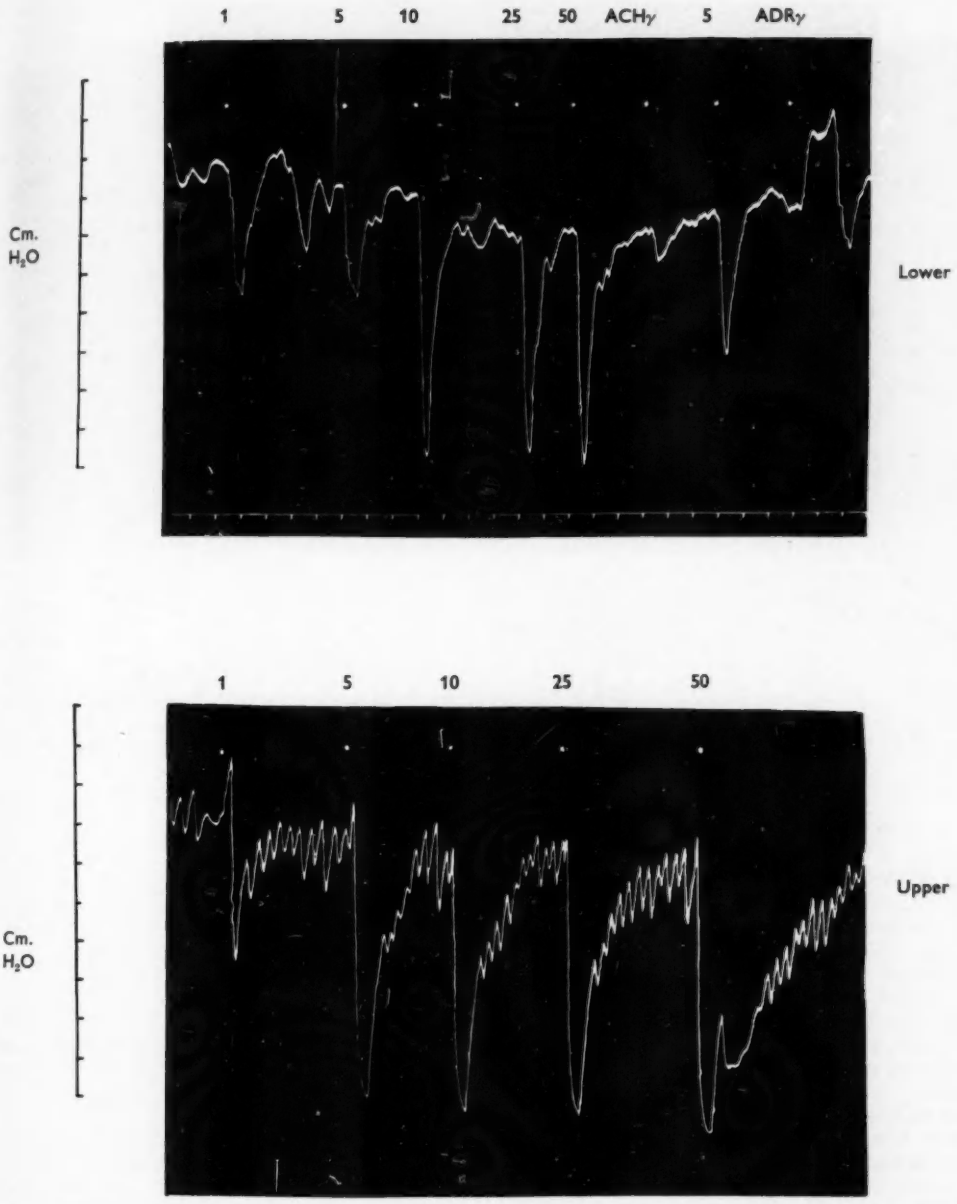

FIG. 4. *Cat, 3·6 kg. male, anaesthetized with chloralose. The upper tracing shows the effect on the isolated sphincter, continuously perfused from the oesophageal aspect, of peripheral vagal nerve stimulation in the neck at frequencies of 1/sec. to 50/sec. for 30 sec. The lower record shows the effects obtained when the direction of perfusion is reversed. The effects of acetylcholine (1 μg. and 5 μg. intravenously) and adrenaline (5 μg. intravenously) are also noted. Pressure changes in cm. H_2O, time in minutes.*

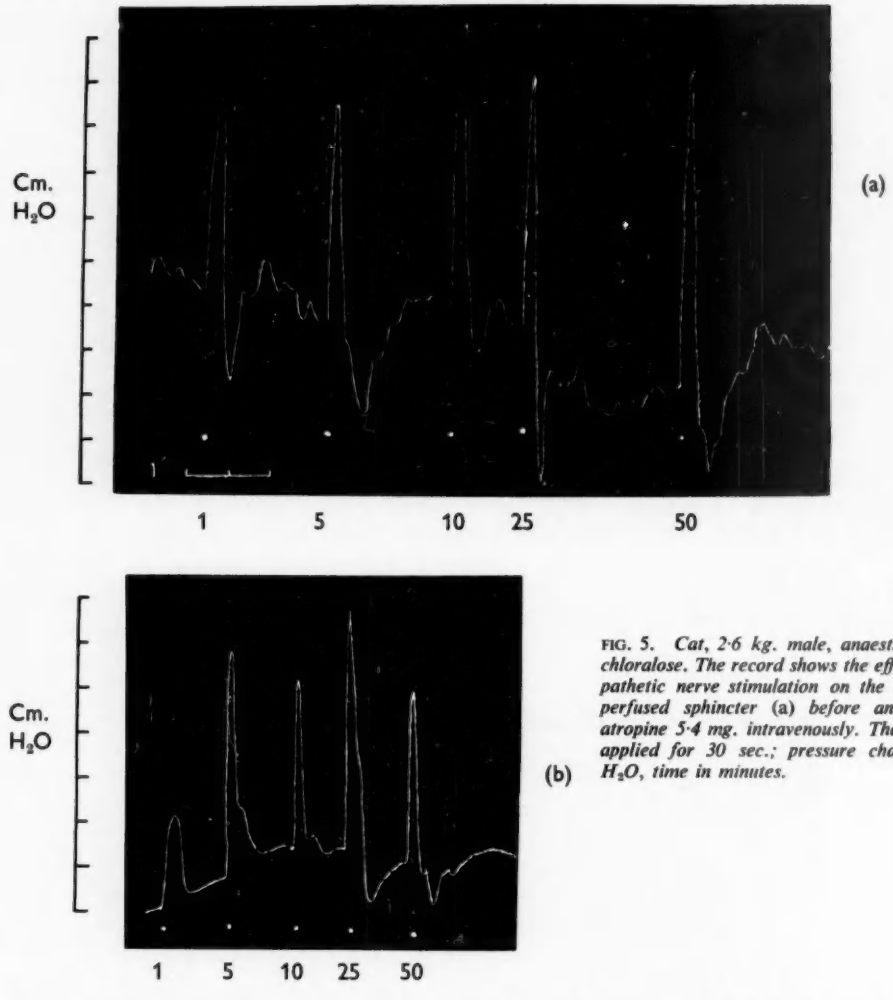

FIG. 5. *Cat, 2·6 kg. male, anaesthetized with chloralose. The record shows the effects of sympathetic nerve stimulation on the continuously perfused sphincter* (a) *before and* (b) *after atropine 5·4 mg. intravenously. The stimulus is applied for 30 sec.; pressure change in cm. H_2O, time in minutes.*

potencies. The sphincter contracts at low dosage (5-10 μg.), and at larger doses a state akin to spasm results. It was also noted that in animals where normal rhythmic movements had become sluggish towards the end of an experiment, either drug would restore a period of brisk alternate contraction and relaxation. Histamine (1-500 μg. intravenously) and 5-hydroxytryptamine (5-500 μg. intravenously) also contracted the sphincter and restored rhythmicity.

Nicotine (50-100 μg. intravenously) produced relaxation of the sphincter (Fig. 6), though a sustained contraction was noted on one occasion. Hexamethonium (10 mg. intravenously) caused a general decrease in tone which recovered within 5 min. and also reduced substantially the effects of nicotine. This was always coincident with the fall in blood pressure, but rhythmic changes of tone of the sphincter were not affected (Fig. 6).

Hyoscine and atropine had little effect on the rhythmic variations in tone of the sphincter, although occasionally after atropine tone was briefly increased. The effect appeared similar to that sometimes seen after vagotomy and was of the same duration. While the relaxation produced by acetylcholine (5 μg. intravenously) could readily be abolished by either hyoscine or atropine (500 μg.

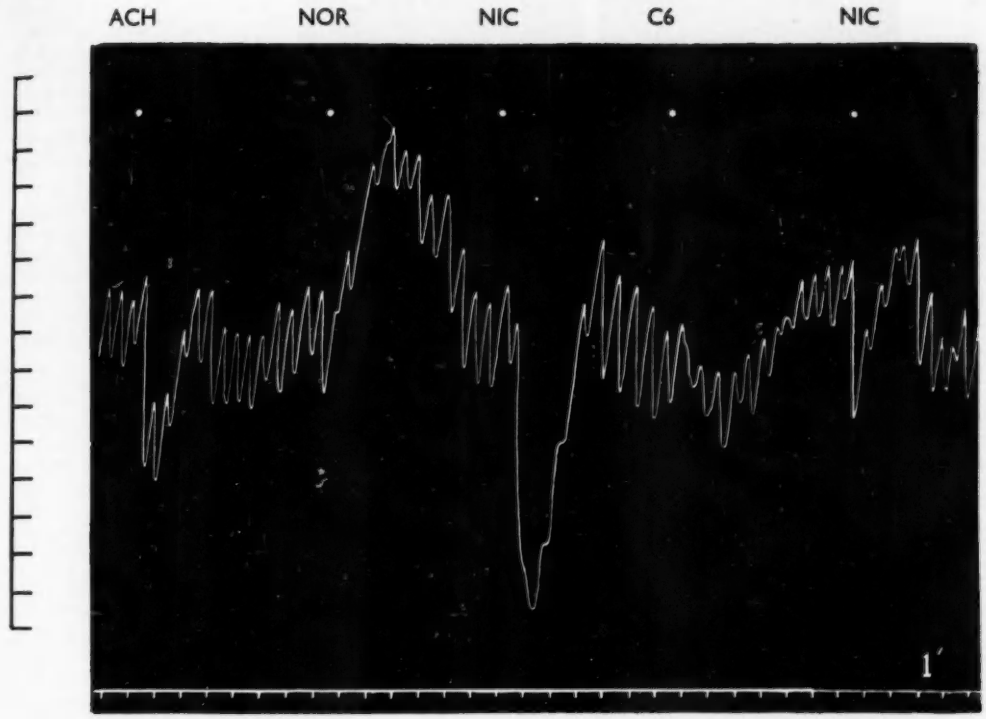

FIG. 6. *Cat, 1·8 kg. female, anaesthetized with chloralose. This record shows the effect of acetylcholine (5 μg. intravenously), noradrenaline (10 μg. intravenously), and nicotine (100 μg. intravenously); hexamethonium (10 mg. intravenously) results in a small decrease in tone, after which the effect of nicotine is considerably reduced. Pressure change in cm. H_2O, time in minutes.*

intravenously), large doses (5-10 mg. intravenously) were required to abolish the effects of vagal stimulation.

Isopropylnoradrenaline (Fig. 7) was the only other drug which produced regular relaxation of the cardia, a dose of 50 μg. intravenously completely abolishing both the sphincteric action and fluctuations in tone for several minutes. The relaxation produced by isopropylnoradrenaline was unaffected by atropine.

OTHER EFFECTS Cannon (1908) postulated the theory of acid control of the cardiac sphincter and we investigated the effects of acid and alkali. If HCl is allowed to flow through the isolated sphincter, there is a gradual increase in tone as the concentration rises from N/100 to N/10. This increase in tone was not affected by bilateral vagotomy. At this concentration the cardia on one occasion, after a brief period of spasm, became completely incompetent and did not recover during the remaining three hours of experiment (Fig. 8).

Weak alkali (N $NaHCO_3$) slightly lowers the tone of the cardia and strong alkali (N/10, NaOH) destroys it irreversibly. Ice-cold saline slightly increases tone. Isopropylnoradrenaline (1-100 μg./ml.) and 5-hydroxytryptamine (50 μg.-1 mg./ml.) added to the perfusate were without effect.

An increase in tone of the sphincter was also seen if N/10 HCl (1-5 ml.) was injected into the saline in the isolated gastric pouch, but it was smaller than when the acid was applied directly to the mucous membrane of the sphincter and developed more slowly. It was unaltered by vagotomy.

DISCUSSION

So many different techniques have been used to study the cardiac sphincter, in so many different

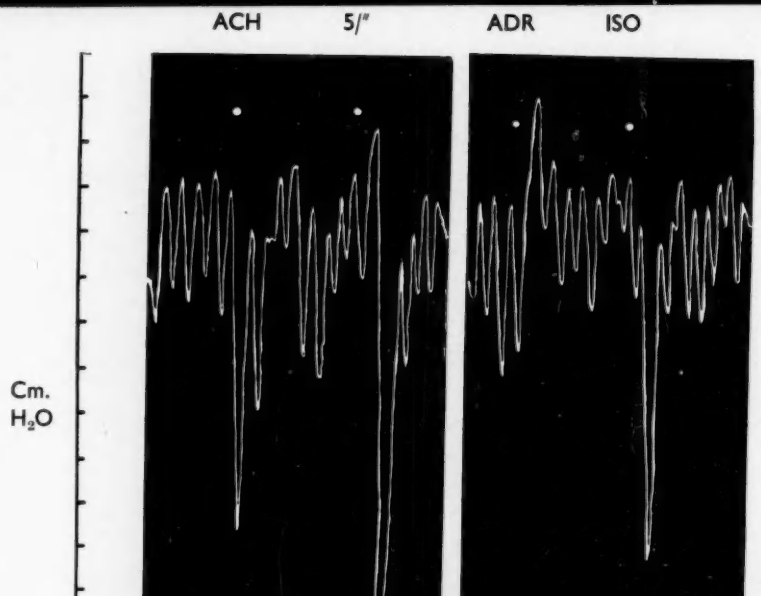

FIG. 7.

FIG. 7. *Cat, 2·8 kg. male, anaesthetized with chloralose. The record shows the effects of acetylcholine (5 μg. intravenously), vagal stimulation (5/sec. for 30 sec.), adrenaline (5 μg. intravenously) and isopropylnoradrenaline (1 μg. intravenously) on the cardia perfused through the lumen with saline. Pressure changes in cm. H_2O, time in minutes.*

FIG. 8. A. *Cat, 3·6 kg. male, anaesthetized with chloralose. The record shows the effect of perfusing the sphincter (isolated) with N/100 HCl. The tone of the sphincter increases during the perfusion and quickly returns to normal when this is stopped.*

B. *Cat, 3·6 kg. male, anaesthetized with chloralose. With N/10 HCl a brief spasm occurs after which the cardia becomes incompetent, and does not recover up to one hour.*

Pressure changes in cm. H_2O, time in minutes. The acid was injected into the side arm of the perfusing apparatus and the apparent delay in onset of the response represents the time taken for the acid to reach the sphincter.

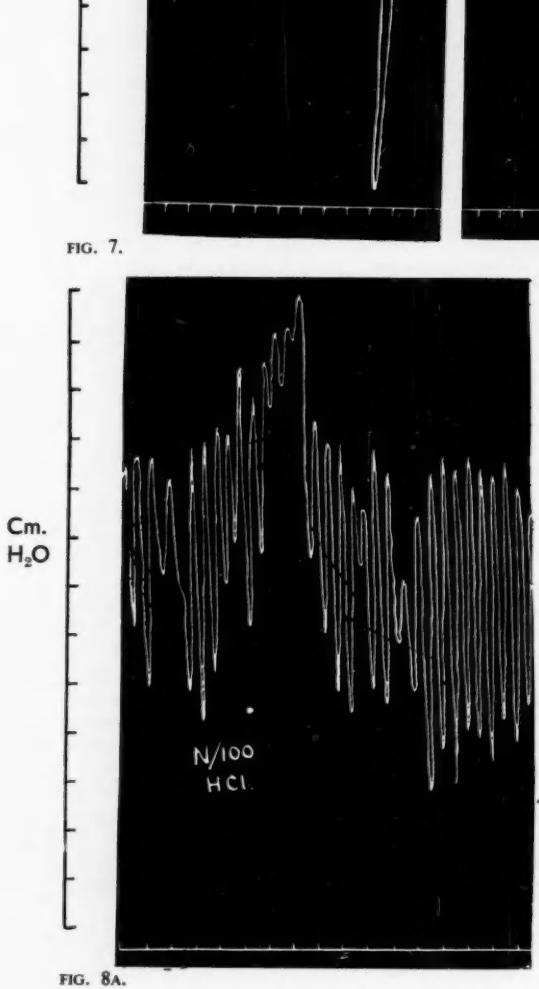

FIG. 8A.

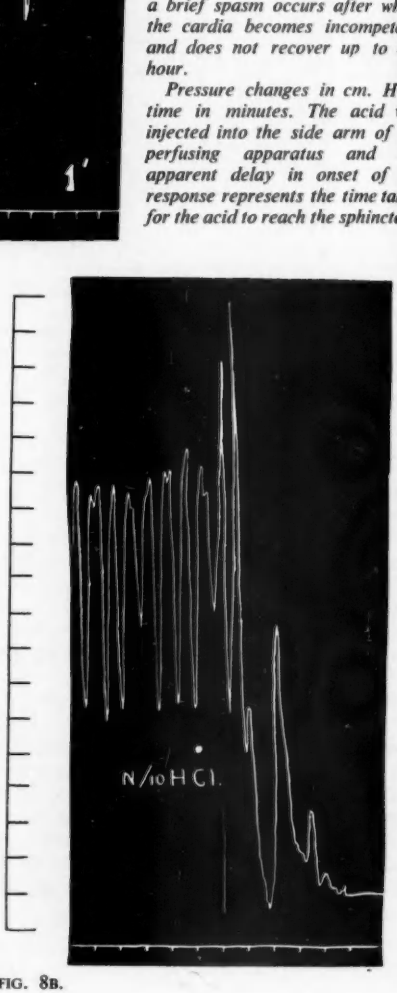

FIG. 8B.

species, that it is difficult to abstract a clear picture of the elements which contribute to and control its function. The anatomy of the lower end of the oesophagus in the cat is similar to that in man; we have therefore tried to assess by simple techniques the effects of various procedures on the functioning cardiac sphincter in the cat.

It was surprising to find that even the region with sphincteric action had not been clearly defined, so our first experiments were directed to this end. Mild distension of the oesophagus showed that the lower 2 cm. remained tightly closed, whereas the rest of the organ expanded. The reactions of this last 2 cm. were also different from those of the body of the oesophagus and from those of the stomach, so that it cannot be said to behave as part of the gastric musculature as suggested by Carlson *et al.* (1922) and by McCrea *et al.* (1925). The region of the sphincter comprises a longitudinal and circular muscle coat with mucous membrane containing the finely distributed muscularis mucosa. This muscularis in the cat contains only longitudinally disposed muscle fibres (Hughes, 1955). Whilst it has been shown that contraction of the longitudinal muscle of the oesophagus accompanies opening of the cardiac sphincter (Torrance, 1958), a substantial reduction in the amount of muscle available for this contraction by separation of the oesophagus from sphincteral region does not decrease the efficiency of the sphincter. The conclusion that the circular muscle fibres of the lower oesophagus are responsible for sphincteric actions seems inescapable, especially as the sphincter is destroyed by an incision dividing these fibres, an effect also shown to be possible in men (Atkinson *et al.*, 1957).

It is clear that the sphincter resists the passage of fluid in either direction and that the 'normal' state of the organ is one of closure. When it is opened, during a continuous flow of fluid, rhythmic fluctuations in diameter can be seen. Immediately after killing the animal, the sphincter becomes completely incompetent.

All of our experiments point to the external nerve supply of the sphincter playing a major part in the control of its function. In the spinal cat, for instance, where reflexes through the higher centres are abolished, the pressure needed to open the sphincter was higher than in anaesthetized animals. Although our experiments have not excluded the possibility of a local reflex mechanism, a central reflex mediated by sensory and motor nerves in the vagi has been demonstrated. Stimulation of either the central or peripheral ends of a vagus nerve leads to dilatation of the sphincter. Distension of the oesophagus leads to the immediate opening of the cardiac sphincter, even when muscular continuity is absent, and this effect is abolished by vagotomy in the neck. A similar reflex in dogs was shown to follow oesophageal distension by Grondahl and Haney (1940) and in man to accompany swallowing (Atkinson *et al.*, 1957). It has also been shown by cineradiography that if contrast medium is introduced into the oesophagus through a Ryle tube, the human cardiac sphincter is able to support a column of fluid about 7 cm. high without leakage, and that on making a 'dry' swallowing movement, the sphincter opens (Ardran, Clark, and Vane, unpublished observations).

Both these observations in humans and our present work in cats make it probable that the valvular nature of the cardiac sphincter, as discussed by Dornhorst *et al.* (1954), is a facet of the reflex control.

The effects of distension of the stomach on the tone of the cardiac sphincter were interesting, both in this respect and in relation to the function of the stomach as a storage organ. Moderate distension of the gastric pouch was accompanied by a tighter closure of the sphincter, but, with further distension, a stage was reached at which the sphincter relaxed. Both of these effects must also be reflex in nature for they are abolished by cervical vagotomy and are too prompt to be mediated by release of a hormone. Presumably, the initial increase in tone of the cardiac sphincter minimizes reflux of food into the oesophagus, but gross distension of the stomach reflexly opens the sphincter as a safety mechanism to prevent rupture of the stomach.

All of these reflexes were mediated through the vagus nerves and this may well be the major function of the vagal innervation. We obtained no evidence for a tonic vagal discharge to the sphincteric region in our experiments. Such a discharge would tend to keep the sphincter partially open but neither vagotomy nor the intravenous injection of atropine or hexamethonium led to more than a very brief increase in tone of the muscle. In fact, the only direct effect vagotomy seemed to have on the function of the sphincter was to increase slightly the resistance to gastric reflux, as was also found by Carlson *et al.* (1922), an effect probably linked with the abolition of the reflexes just discussed.

In only two of the many experiments did the integrity of the diaphragm appear to augment the sphincteric action and this was by aiding the prevention of gastric reflux. The more general lack of change of function of the sphincter on removal of the diaphragm agrees with the results of Botha (1958b), who concluded that in man the diaphragm might occasionally assist in preventing reflux but was not normally responsible for doing so. In our experiments, the two exceptions might have been

brought about by inadvertent interference with the extrinsic innervation to the sphincter, but it should be mentioned that Shenk and Frederickson (1958) concluded that in the cat the diaphragm considerably augmented the ability of the sphincter to resist passage of fluid.

Direct nerve stimulation confirmed that the vagus supplies predominantly inhibitory and the sympathetic predominantly excitatory fibres to the sphincter. Mixed effects on vagal stimulation were only seen at low frequencies and may have been due either to the inclusion of a few muscle fibres from the stomach or oesophagus (which are contracted by vagal stimulation) or to a mixture of adrenergic and cholinergic nerves in the vagus, as suggested by Paton and Vane (1956). Either interpretation would be in accord with the views of McCrea *et al.* (1925), who found that the effect of vagal stimulation was dependent upon the resting tone of the sphincter. In a muscle with a low tonus, excitatory effects would show up best, but if the muscle is already contracted relaxation would predominate. Although Langley (1898) suggested that the nerves causing relaxation of the sphincter passed intramurally down the oesophagus and Lehmann (1945) confirmed this suggestion in the dog, there is no doubt that in the cat stimulation of the vagus produces relaxation of the cardiac sphincter irrespective of muscular continuity with the rest of the oesophagus.

The experiments with injected drugs all confirm the well-established views on the transmitters of nervous effects. Intravenous acetylcholine reproduced the inhibitory effects of vagal stimulation and atropine or hyoscine blocked both, though more was needed to block the vagal effects, a characteristic common to other sites in the gastrointestinal tract (Ambache, 1955). Similarly, the effects of sympathetic stimulation were mimicked by both adrenaline and noradrenaline, but not by isopropylnoradrenaline.

Finally, it was shown that a mechanism exists whereby acid can induce an increase in tonus in the cardiac sphincter when applied either to the mucosa of the stomach, as suggested by Cannon (1908), or to the mucous membrane of the sphincteric region itself. The mechanism by which this effect is brought about is uncertain.

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REFERENCES

Ambache, A. (1955). The use and limitations of atropine for pharmacological studies on autonomic effectors. *Pharmacol. Rev.*, **7**, 467-494.

Atkinson, M., Edwards, D. A. W., Honour, A. J., and Rowlands, E. N. (1957). Comparison of cardiac and pyloric sphincters. *Lancet*, **2**, 918-922.

Barrett, N. R. (1954). Hiatus hernia. *Brit. J. Surg.*, **42**, 231-243.

Botha, G. S. Muller (1958a). A note on the comparative anatomy of the cardio-oesophageal junction. *Acta anat.* (*Basel*), **34**, 52-58.

—— (1958b). Mucosal folds at the cardia as a component of the gastro-oesophageal closing mechanism. *Brit. J. Surg.*, **45**, 569-580.

Cannon, W. B. (1908). The acid closure of the cardia. *Amer. J. Physiol.*, **23**, 105-114.

Carlson, A. J., Boyd, T. E., and Pearcy, J. F. (1922). Studies on the visceral sensory nervous system. XIV. *Arch. intern. Med.*, **30**, 409-433.

Dornhorst, A. C., Harrison, K., and Pierce, J. W. (1954). Observations on the normal oesophagus and cardia. *Lancet*, **1**, 695-698.

Fyke, F. E. Jr., Code, C. F., and Schlegel, J. F. (1956). The gastro-oesophageal sphincter in healthy human beings. *Gastroenterologia* (*Basel*), **86**, 135-150.

Grondahl, J. W., and Haney, H. F. (1940). Attempt to produce experimental cardiospasm in dogs. *Proc. Soc. exp. Biol.* (*N.Y.*), **44**, 126-129.

Hughes, F. B. (1955). The muscularis mucosae of the oesophagus of the cat, rabbit, and rat. *J. Physiol.* (*Lond.*), **130**, 123-130.

Klee, P. (1919). Beiträge zur patholegeschen Physiologie der Magerinnervation. *Dtsch. Arch. klin. Med.*, **129**, 204-241.

Knight, G. C. (1934). The relation of the extrinsic nerves to the functional activity of the oesophagus. *Brit. J. Surg.*, **22**, 155-168.

Koennecke, W. (1922). Experimentelle Innervationsstörungen am Magen und Darm. *Z. ges. exp. Med.*, **28**, 384-412.

Kosterlitz, H. W., Krayer, O., and Matallana, A. (1954). The effect of moderately large doses of veratramine and veratrosine on the rhythm of the acutely denervated heart of the cat. *J. Physiol.* (*Lond.*), **124**, 40P.

Langley, J. N. (1898). On inhibitory fibres in the vagus to the end of the oesophagus and stomach. *Ibid.*, **23**, 407-414.

Lehmann, G. (1945). Gastric cardiospasm in the dog. *Amer. J. Physiol.*, **143**, 163-168.

Lendrum, F. C. (1937). Anatomic features of the cardiac orifice of the stomach, with special reference to cardiospasm. *Arch. intern. Med.*, **59**, 474-511.

May, W. P. (1904). The innervation of the sphincters and musculature of the stomach. *J. Physiol.* (*Lond.*), **31**, 260-271.

McCrea, E. D., McSwiney, B. A., and Stopford, J. S. B. (1925). Effects of section of the vagi nerves on the motor activity of the stomach. *Ibid.*, **60**, XXIX-XXX.

Nauta, J. (1955). *Een studie van het afsluitings—mechanisme tussen slokdarm en maag*. Stenfert Kroese N.V., Leiden.

—— (1956). The closing mechanism of the cardia. *Arch. chir. neerl.*, **8**, 280-291.

Paton, W. D. M., and Vane, J. R. (1956). The responses of the isolated stomach of a guinea-pig to electrical excitation. Proc. XX Int. Physiol Congress, p. 709.

Roux, J. (1939). Le segment cardio-oesophagien. *Bronchoscopie*, pp. 81-113.

Shenk, E. A., and Frederickson, E. L. (1958). Oesophageal sphincter mechanisms. *Fed. Proc.*, **17**, 142.

Torrance, H. B. (1958). Studies on the mechanism of gastro-oesophageal regurgitation. *J. roy. Coll. Surg. Edinb.*, **4**, 54-62.

Veach, H. O. (1925). Studies on the innervation of smooth muscle. III. Splanchnic effects on the lower end of the oesophagus and stomach of the cat. *J. Physiol.* (*Lond.*), **60**, 457-478.

—— (1926). Studies on the innervation of smooth muscle. IV. Functional relations between the lower end of the oesophagus and stomach of the cat. *Amer. J. Physiol.*, **76**, 532-537.

Gut, 1961, **2**, 263

Serum studies in man after administration of vitamin A acetate and vitamin A alcohol

I In normal subjects

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In 1934, Chesney and McCoord reported poor absorption of vitamin A in coeliac disease. Many papers have since been published on the use of vitamin A absorption tests as an index of small intestinal function. The absorption of fat-soluble vitamins is related to the absorption and transport of fats, since both are insoluble in water and are soluble in the same solvents (Clausen, 1943; Sobel, 1952). Vitamin A is absorbed in the upper small intestine as vitamin A alcohol, followed by reconversion to an ester before it reaches the circulation. The three main factors for its normal absorption are: 1, bile salts, 2, intestinal motility, and 3, pancreatic secretion (Gray, Morgareidge, and Cawley, 1940; Clausen, 1943). These factors are also necessary for the proper absorption of a neutral fat. After administration of a large dose of vitamin A orally both lymphatic and serum levels of vitamin A are raised, the greater in the lymphatic circulation (Eden and Sellers, 1949). Studies on a patient with a diverted thoracic duct have shown that vitamin A appears in increased amounts in thoracic duct lymph after oral administration of a large dose (Drummond, Bell, and Palmer, 1935). If post-absorptive serum curves are to be used as a test of intestinal absorption in disease it is important that the factors which affect the curves in normal controls should be understood. Hillman and Becker (1957) suggested that the vitamin A absorption test was of limited value, a conclusion principally based on differences between repeat tests carried out on individuals and groups under varying conditions and relying on a four-hour sample to test for absorption and using amounts from 50,000 to 150,000 units as a test dose of vitamin A.

It has long been known that many factors may affect vitamin A absorption curves. It is generally accepted that in patients with either normal or abnormal absorption, aqueous dispersions of vitamin A produce (*a*) higher liver storage levels, (*b*) higher serum levels, and (*c*) lower faecal excretion than do oily preparations (Lewis, Bodansky, Birmingham, and Cohlan, 1947; Kramer, Sobel, and Gottfried, 1947; May and Lowe, 1948; Barnes, Wollaeger, and Mason, 1950). That the relation of peak to pre-test levels of serum vitamin A should be used as an index of intestinal absorption has been suggested by Mendelhoff (1954), and he considered that a rise to four times the fasting level indicated normal absorption. The importance of taking a meal during the test was demonstrated in Mendelhoff's paper, and later by Hillman and Becker (1957), who found that fatty meals gave the sharpest rise in the serum curve.

In this paper we propose to discuss the effect of these factors and show the effect of diet and exercise on post-absorptive curves of normal controls.

MATERIAL AND METHODS

Double vitamin A absorption tests were carried out using either the vitamin A acetate or vitamin A alcohol, with an interval of at least 48 hours between the two tests. The vitamin A preparation was dissolved in arachis oil (1 ml. containing 250,000 i.u.) and was sealed in nitrogen in a glass ampoule. No emulsifiers were used as these may produce normal absorption curves even in malabsorptive states (Fox, 1949).

Normal subjects were studied, resting without a fatty meal and with a fatty meal, and ambulant without a fatty meal and with a fatty meal. These controls were residents, medical students, and other medical personnel, and some patients under investigation for conditions not related to the alimentary tract. Their ages ranged from 20 to 60 years, the majority being men between 20 and 30 years of age. There was no modification in their diet before the tests and no extra vitamin A was given before the test. Tests were carried out on 27 subjects (18 men and nine women).

The subjects were allowed only fluids on the morning of the test. At zero time, usually 9.0 a.m., 5 ml. blood was withdrawn into a dry tube. The subject was then given 250,000 i.u. of the vitamin A preparation in 200 ml. milk. One to two hours later some subjects were given a mixed meal containing 50 g. fat. Blood was withdrawn at three and five hours from zero. Serum vitamin A and serum

carotene were estimated simultaneously by the Carr-Price technique as modified by Kimble (1939). Values were corrected for carotene by the factor (0·4) of Moore (1957), who also described the chemical part of the test.

RESULTS

In Table I and Fig. 1 are shown the results of estimation of vitamin A post-absorption peaks and also the fasting carotene levels in 31 tests carried out on normal subjects. Fasting serum vitamin A levels varied from 83 to 333 i.u.% and the mean peak figure for vitamin A acetate absorption was 1,231 i.u.%, with a range of 465 to 1,803 i.u.%; for vitamin A alcohol the mean peak was 1,428 i.u.% with a range of 875 to 2,592 i.u.%. The medians were 1,205 i.u. % and 1,140 i.u.% respectively. All, except subjects 9 and 10, had a fatty meal during the test. Fasting serum carotene levels in our series varied from 80 to 320 μg.% in over 30 normal controls, many of whom are not included in this series as they were not studied with both vitamin preparations.

Subject No. 1 had three tests carried out in the space of 10 days, the highest figure resulting from the third test. Subject No. 3 was nauseated during the test which may explain the low figures. The absorption curve does not appear to be related to the fasting vitamin A level. In 30 tests carried out using three, five, and eight-hour samples the peak was at three hours in 15, at five hours in 14, and at eight hours in one, this last probably being due to a delay in giving the subject a meal after the test dose. Furthermore, in 30 tests carried out on abnormal subjects the same time relation was noted as regards peak figures. From this we can conclude that in 96·7% of cases samples at three and five hours should detect peak figures.

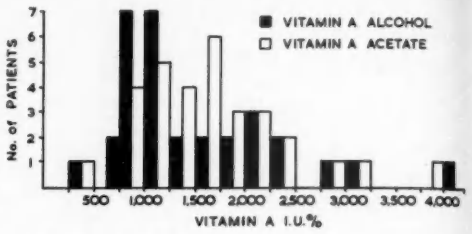

FIG. 1. *Frequency distribution of post-absorption peaks of vitamin A acetate and vitamin A alcohol (dose 250,000 i.u. vitamin): 31 tests in normal controls.*

EFFECT OF FOOD ON PEAK In Table II the curves in subjects with and without a fatty meal during the test are compared.

As can be seen, six of these controls showed post-absorption peaks, without a fatty meal, which would be judged as quite abnormal and indicating mal-absorption; however, the administration of a fatty meal during the test corrected this error in all except one subject who was nauseated during the test.

EFFECT OF EXERCISE ON PEAK We noted many low curves in our ambulant subjects, so these tests were compared with those of resting subjects.

As can be seen from Table III, Cases 3 and 4 in the ambulant group showed unusually low curves. The mean rise in the ambulant group was lower than that for the resting group. While it has been suggested that exercise might have an effect on these curves no figures have been previously reported. There are not sufficient results for a reliable statistical analysis ($P = 0{\cdot}05$).

TABLE I

RESULTS OF VITAMIN A ABSORPTION TESTS IN NORMAL ADULT SUBJECTS USING 250,000 I.U. OF VITAMIN A ACETATE OR ALCOHOL PREPARATION

Case No	*Sex*	*Fasting Carotene (mg.%)*	*Vitamin A Acetate (i.u.%)*			*Vitamin A Alcohol (i.u.%)*	
			Fasting	*3 Hours*	*5 Hours*	*3 Hours*	*5 Hours*
1	M	144	290	1,300	1,000	1,000 2,592[1]	475 1,150
2	M	185	110	1,210	750	875	575
3	F	230	83	465	215	2,085	661
4	M	—	150	1,070	450	1,050	450
5	M	190	190	Not done	Not done	1,420	750
6	M	185	190	1,020	460	1,120	350
7	F	237	178	1,803	1,828	1,690	2,327
8	F	156	250	1,600	1,460	1,600	950
9	M	—	220	1,100	—	1,150	1,350
10	M	100	333	1,516	1,499	1,131	1,089
Mean		178	199	1,231	958	1,428	830
Range		100-237	83-333	465-1,803	215-1,828	875-2,592	350-2,327
Median		185	190	1,205	750	1,140	618

[1]This was the third test carried out over a 10-day period on this subject.

TABLE II

PEAK SERUM LEVEL OF VITAMIN A AFTER ADMINISTRATION OF 250,000 i.u.% WITHOUT AND WITH FATTY MEAL

No.	*Sex*	*Without Fatty Meal*		*With Fatty Meal*	
		Vitamin Preparation	*Peak Figure*[1]	*Vitamin Preparation*	*Peak Figure*
1	M	Alcohol	135	Alcohol	988
		Acetate	296	Alcohol	1,136
				Alcohol	2,592
				Acetate	1,340
2	M	Alcohol	575	Alcohol	870
		Acetate	700	Acetate	1,249
3	F	Alcohol	480	Alcohol	2,055
		Acetate	295	Acetate	460
4	M	Alcohol	1,050	Alcohol	1,080
5	F	Alcohol	750	Alcohol	1,420
6	M	Alcohol	450		
		Alcohol	480		
7	M	Alcohol	320		
		Alcohol	731		
Mean			521		1,320
Median			480		1,136
Range			125-1,050		460-2,592

[1]Peak expressed as actual maximal rise from fasting level in i.u.%.

TABLE III

PEAKS OF VITAMIN A ABSORPTION TESTS IN RESTING AND AMBULANT SUBJECTS

No.	*Sex*	*Resting Vitamin A (i.u.%)*	*Ambulant Vitamin A (i.u.%)*
1	M	1,250	—
2	F	1,200	350
3	M	1,014	89
4	F	2,175	330
5	M	2,522	1,350
6	M	960	1,249
7	F	1,377	465
8	F	1,859	2,175
9	M	963	1,080
10	M	1,420	1,420
Mean		1,475	912
Range		963-2,522	30-2,175
Median		1,314	1,080

DISCUSSION

There is still some difference of opinion as to the relative value of the tests used in assessing intestinal absorption.

The fat balance test is mainly affected by absorptive capacity. The vitamin A test is affected by many factors other than absorption. Emulsification has been shown to increase the serum absorption curve and reduce the faecal excretion of the vitamin in abnormals as well as in normal subjects (Fox, 1949; Barnes *et al.*, 1950). The difference in absorption between emulsified and non-emulsified preparations is more evident in patients with malabsorption. When the particles are of small size in a water-soluble phase further emulsification may not be needed (May and Lowe, 1948). In preliminary tests of absorptive function emulsifiers such as Tween 80 should be excluded from preparations used in absorptive tests, unless one is specifically looking for an emulsification defect (lack of bile) as distinct from a digestive (pancreatic or succus entericus) or absorptive (intestinal wall) defect.

The differential absorption of vitamin A alcohol relative to that of vitamin A esters, such as vitamin A acetate, has been previously noted by Lewis *et al.* (1947), Kagan, Thomas, Jordan, and Abt (1950), and by Barnes *et al.* (1950). They concluded that vitamin A alcohol was better absorbed than the esterified form in normal and in abnormal human subjects. Our present findings suggest that there is little differential absorption of the two forms of the vitamins in normal subjects.

The dose used by various authors has shown such a variation that it is difficult to compare results from different papers. The dosage ranged from 90,000 to 600,000 i.u., some doses being related to body weight. Mendelhoff (1954) found a rise of over 100 i.u.% following 90,000 to 180,000 i.u. taken orally. Kramer *et al.* (1947) had noted a rise of over 4,000 i.u. following 6,000 i.u./lb. body weight. In our experience 250,000 i.u. is a satisfactory dosage in terms of body weight when dealing with concentrated preparations as small errors in volume could mean up to 100,000 i.u. difference in dosage. This dosage is equivalent to 3,571 i.u./kg. or 1,630 i.u./lb. in a 70 kg. subject. Dosage up to 150,000 i.u. may be taken up too rapidly by the tissues so that good absorption curves do not result; however, with dosage over 200,000 i.u. a good curve usually results in normal subjects.

Mendelhoff (1954) considered that a rise to four times the fasting vitamin A level indicated normal absorption but, for a number of reasons, he held that one should not evaluate the normality of a curve from the relation of post-absorptive level to fasting level. As pointed out by Popper, Steigman, Mayer, and Zevin (1943) the fasting level may be an index of vitamin A storage rather than absorption and they concluded that the curve was not necessarily related to the fasting level. It should be remembered that the chemical estimation of vitamin A is very much affected by the carotene level since the factor of 0·4 times the carotene figure in milligrams per cent, as suggested by Moore (1957), must be subtracted from the final vitamin A figure in order to correct for the blue colour produced by carotene with the antimony trichloride. At levels of carotene over 300 mg.% the correction of 0·4 is probably excessive and a smaller

TABLE IV

VARIATIONS IN DOSE OF VITAMIN A USED FOR INVESTIGATIONS BY DIFFERENT AUTHORS

Author	*Dose (i.u.)*	*Expected Peak in Normal Subjects (i.u.%)*
Adlersberg and Sobotka (1943)	(a) 90,000 (b) 80,000 (c) 600,000 (occasionally)	53% rise for (a) and (b)
Kramer, Sobel and Gottfried (1947)	6,000/lb. body weight	4,000
Mendelhoff (1954)	100,000	4 times fasting level or 100 μg.%
Week and Sevigne (1950)	440,000	Over 2,000
Kagan, Thomas, Jordan and Abt (1950)	5,000 /lb. body weight	2,480-2,120
Barnes, Wollaeger, and Mason (1950)	7,500	500
This paper	250,000	800-4,000

factor (0·3 or even 0·25) might be more accurate. Paterson and Wiggins (1954) considered that if the serum level rose to over 500 i.u. after administration of 250,000 i.u. of vitamin A steatorrhoea was almost certainly excluded. As seen in Table I, the lowest fasting vitamin A level was found in a subject with nearly the highest carotene figure (fasting vitamin A, 83 i.u.%, fasting carotene, 230 μg%).

A meal definitely raised the absorption curves, as shown in Table II, and already pointed out by Hillman and Becker (1957). These authors noted that fatty meals gave the best response. We have relied on meals containing 50 g. fat given one to two hours after vitamin A dosage. In Table II it is shown that there is a rise in absorption from a mean level of 531 i.u. without a fatty meal to 1,320 i.u. when a fatty meal is added during the test. Many tests which were carried out with the patient fasting right through the test showed abnormally low curves but on adding the fatty meal during the test the curves became quite normal. A possible explanation for this difference is that the small volume in which the vitamin A is given is not sufficient to stimulate secretion of digestive enzymes, or an added force is needed to drive the absorbed vitamin A into the lacteal and systemic circulations (Mendelhoff, 1954).

The effect of exercise on the curve is not generally appreciated, though Mendelhoff (1954) did discuss the possible effect. We have noted that vitamin A tests carried out on ambulant controls have frequently shown lower absorption curves than those in resting controls. This difference is shown in Table III. It is possible that many of the unsatisfactory results obtained in controls by Hillman and Becker (1957) were related to the fact that their controls were mainly medical personnel in the course of their duties.

Vitamin A absorption curves from the literature show much variance as a result of the factors mentioned. A coincidental meal must be given and exercise must be avoided in all tests, as it has been shown that excessive variations occur if the patients are kept fasting during the test or if exercise is allowed.

SUMMARY

The absorption of vitamin A alcohol and acetate in normal subjects is discussed.

There is no significant difference between the serum level after the administration of vitamin A alcohol and vitamin A acetate.

The use of a fatty meal during the test is recommended.

Exercise appears to affect the curve; it is important to keep subjects resting during the test.

The use of two samples (at three and five hours) is more accurate in detecting peak serum absorption figures than one sample at four hours.

One of us (J.J.F.) wishes to acknowledge with thanks a personal grant from the Medical Research Council of Ireland which has also contributed towards the general expenses of the study. Professor E. J. Conway, F.R.S., is to be thanked for hospitality and use of apparatus. We are much indebted to Messrs. Roche Products for generous supplies of the vitamin A preparations used in these tests.

REFERENCES

Adlersberg, D., and Sobotka, H. (1943). *Gastroenterology*, **1**, 357.
Barnes, B. C., Wollaeger, E. E., and Mason, H. L. (1950). *J. clin. Invest*, **29**, 982.
Chesney, J., and McCoord, A. B. (1934). *Proc. Soc. exp. Biol. (N.Y.)*, **31**, 887.
Clausen, S. W. (1943). *Harvey Lect.*, **38**, 199.
Drummond, J. C., Bell, M. E., and Palmer, E. T. (1935). *Brit. med. J.*, **1**, 1208.
Eden, E., and Sellers, K. C (1949). *Biochem. J.*, **44**, 264.
Fox, H. J. (1949). *J. Lab. clin. Med.*, **34**, 1140.
Gray, E. L., Morgareidge, K., and Cawley, J. D. (1940). *J. Nutr.*, **20**, 67.
Hillman, H., and Becker, N. J. (1957). *Gastroenterology*, **32**, 738.
Kagan, B. M., Thomas, E. M., Jordan, D. A., and Abt, A. F. (1950). *J. clin. Invest.*, **29**, 141.
Kimble, M. S. (1939). *J. Lab. clin. Med.*, **24**, 1055.
Kramer, B., Sobel, A. E., and Gottfried, S. P. (1947). *Amer. J. Dis. Child.*, **73**, 543.
Lewis, J. M., Bodansky, O., Birmingham, J., and Cohlan, S. Q. (1947). *J. Pediat.*, **31**, 496.
May, C. D., and Lowe, C. U. (1948). *J. clin. Invest.*, **27**, 226.
Mendelhoff, A. L. (1954). *Ibid*, **33**, 1015.
Moore, T. (1957). *Vitamin A*. Elsevier, Amsterdam.
Paterson, J. C. S., and Wiggins, H. S. (1954). *J. clin. Path.*, **7**, 56.
Popper, J., Steigman, Mayer, K. A., and Zevin, S. S. (1943). *Arch. intern. Med.*, **72**, 439.
Sobel, A. E. (1952). *Vitam. and Horm.*, **10**, 47.
Week, E. F., and Sevigne, F. J. (1950). *J. Nutrit.*, **40**, 563.

Gut, 1961, **2**, 267

The problems of closed liver injuries[1]

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SYNOPSIS Because laceration of the liver is a condition which the surgeon is rarely called upon to treat, 36 cases of liver injury admitted between 1926 and 1958 have been reviewed and the results compared with those of other authors. As well as discussing the incidence of this injury and its relation to age, sex, and pathology, the surgical management is outlined. The greatest single factor which has assisted the surgeon is transfusion of whole blood. Early operation is considered advisable, and suturing is preferred in repairing damage to the liver substance and achieving haemostasis. Nevertheless, the mortality rate is high, in this series 60%, which compares favourably with that of other series. It is notable that patients operated on have a better chance of survival than those not operated upon.

Hepatic trauma due to external violence is not common, but is a condition which is associated with a high mortality rate. Apart from tears sustained in the course of difficult cholecystectomies, laceration of the liver due to external trauma is a condition which the average surgeon is rarely called upon to treat. Because of this, not only does one find that views in regard to the local management of the injured organ vary, but there is little known about such problems as incidence, complications, mortality rates, and treatment.

In order to try to build up some authoritative picture of this type of injury, a review of 36 cases of liver injury admitted to the Cardiff Royal Infirmary and from hospitals in four heavily industrialized mining valleys radiating from the northern boundaries of the city was undertaken. In this particular review, which covers the period 1926-58, there were 30 non-penetrating injuries confirmed either at operation or at necropsy, five presumed cases, and one of open injury.

INCIDENCE

The assessment of incidence was found to be difficult, for there were many years in which no cases were seen and information on what percentage of admissions were emergencies was not recorded. However, between 1949 and 1953 the incidence in the Cardiff Royal Infirmary was found to be 1 in 4,000, but in one valley (Pontypridd) during the years 1955-57, the incidence was 1 in 1,200. The latter figures compare closely with those of Edler (1887) and Thorlakson and Hay (1929), who in each case gave a figure of 1 in 1,300, whereas Wright, Prigot, and Hill (1947) found 27 cases occurring in 34,000 cases admitted to the Harlem Hospital between 1953 and 1945, a figure of 1 in 1,259.

The problems of closed splenic and renal injuries are well described in most surgical textbooks, but little is said of rupture of the liver. Because descriptions of the two former injuries are accompanied either by historical annotations or by profusely coloured illustrations, the focus of interest tends to be centred on those two organs and the impression is conveyed that they are the commonest organs to be injured in the abdominal cavity. This is far from the truth, for in this series during the only period in which complete statistics were available from all the areas under consideration, there were 11 hepatic compared with 12 splenic and 17 renal injuries. Below are summarized figures from past reviews compared with the present series, and it is surprising to see that, apart from the figures of Hinton (1929) and Clarke (1954), in all other series the liver appears to be more frequently damaged than the spleen.

TABLE I

	Liver	*Spleen*	*Kidney*	*Pancreas*	*Gastro-intestinal Tract*
Present review	11	12	17	1	9
Vance (1928)	59·9%	33%	21·5%	4·4%	18·2%
Hinton (1929)	7	8	18	—	12
Allen (1931)	24	10	—	—	41
Lewis and Trimble (1933)	20	12	30	—	39
Clarke (1954)	8	27	10	1	5

[1]Synopsis of a thesis accepted by the University of Cambridge in 1959 for the degree of M.D.

AGE, SEX, AND AETIOLOGY

Of the 30 cases recorded in this review 24 males and six females were injured, a ratio of four to one. The age distribution is of interest for predominantly liver injury is an injury sustained by the young (Fig. 1).

In the present review road accidents accounted for eight cases, crushing injuries nine, direct blows on the abdomen six, falls from a height five, 'scissors' type two. In children, road accidents and dangerous play (falls from walls or trees) accounted for many cases, but over the age of 20, although road accidents contribute to the overall figure, injury sustained in dangerous occupations is the most frequent cause. The commonest industrial injury occurred in the mines where men were crushed by derailed coal trams or buried under falls of coal. In so far as the American literature is concerned, all reviews dealing with the topic of abdominal injuries (Thorlakson and Hay, 1929; Allen, 1931; O'Neill, 1941; Papen and Mikal, 1950; Mikal and Papen, 1950; Musselman, Glas, and Campbell, 1955) showed that automobile accidents were by far the commonest cause.

The force of injury required to produce liver injury varies tremendously, from simple to the most major injuries. From an aetiological point of view closed rupture of the liver can be classified as in Table II.

There is no doubt, however, that major trauma is the causative factor in well over 99% of cases. Minor trauma is of importance where there is pathological or physiological enlargement of the liver, as in toxaemia of pregnancy or in the newborn. Spontaneous rupture is a rarity and a most doubtful entity. In all the literature studied I have only found 10 such cases. This paper deals with the type of injury most frequently seen in clinical practice, the type normally associated with trauma of varying degree, but in which there is no underlying hepatic disease.

PATHOLOGY AND COMPLICATIONS

The mechanism of liver rupture is difficult to understand for it is almost impossible to produce experimentally the type of injury seen at operation. Whatever mechanism is involved is really only of academic importance so long as it is clearly understood that the surface laceration is merely the

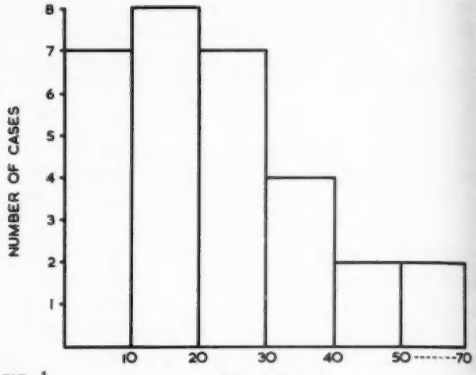


FIG. 1

external evidence of generalized intrahepatic damage of unknown and varying extent, and the whole course and complications of the illness are determined by this latter factor. Three separate systems must be considered, each with its own particular problems.

DIVISION OF INTRAHEPATIC BLOOD VESSELS Haemorrhage undoubtedly constitutes the principal threat to life. A feature which is not fully realized is that the hepatic venous system is of comparable size to the portal and that tributaries of no mean diameter ramify through all parts of the liver substance. Furthermore, the point of entry of the hepatic veins in the vena cava is extremely close, within 1 in. of the right auricle. In effect, therefore, division of a hepatic vein means that these patients are virtually bleeding from the right side of the heart. It is probable that in a very high proportion of those cases in which sudden death occurred at the site of the accident it is these vessels which were torn. The percentage of patients who die at the site of the accident is difficult to determine, but Vance (1928) gave a figure of 33·3%, Lamb (1939) of 43·33%, and in one particular area between 1954 and 1958 in this series the figure was 50%.

INTRAHEPATIC CELLULAR DAMAGE This may be diffuse and varies tremendously in extent. In two cases in this series, in which a small laceration was

TABLE II

Traumatic		*Spontaneous*	
Major	Road accidents, falls from heights, crushing and buffer injuries, etc.	('Apoplexy of the liver')	(1) Without underlying liver disease (2) With underlying liver disease
Minor	(1) Without underlying liver disease Newborn (2) With underlying liver disease Pregnancy		

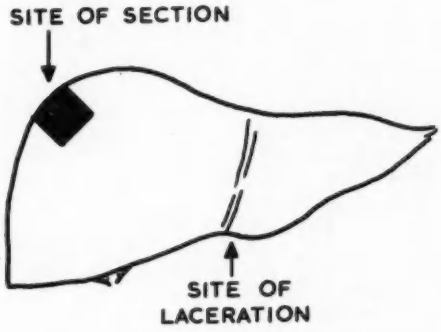
FIG. 2. *Histological section of liver taken at a distance from the site of laceration, demonstrating small haemorrhagic areas.*

found, scattered throughout the liver substance were multiple areas of intrahepatic haemorrhage (Fig. 2). Apart from presenting as a cause of sudden death and the common type met in clinical practice, liver injury may exceptionally present as delayed rupture or later manifestations. The mechanisms can be readily related to the underlying intrahepatic injury:

Subcapsular → Abdominal mass → Rupture into peritoneal cavity
(1) Haematoma
Central
Becomes infected → Liver abscess → Subphrenic abscess
→Communicates with → 'Traumatic haemobilia'
biliary tract
(2) Traumatic bile cyst → Presenting as an abdominal mass
→ Enlarging beneath the diaphragm, dyspnoea
→ Rupture into peritoneal cavity

Should a subcapsular haematoma form it may enlarge in such a fashion as to present as an abdominal mass which subsequently ruptures. On the other hand, a central injury may be produced. Should this in the main be a haematoma, it may increase in size and with a varying amount of bile extend to the surface, or it may form a true bile cyst. If a central haematoma becomes infected, a liver abscess will result which commonly manifests itself at a later stage as a subphrenic abscess. Occasionally, such a haematoma communicates with a major radical of the biliary tree and the contents are discharged into the duodenum and melaena results (traumatic haemobilia).

The intrahepatic post-operative complications are



related to the extent of infarction of liver tissue Thus:

Loss of blood supply → Infarction → Necrosis → Sequestration
Subphrenic abscess ← Liver abscess ← Infection → Secondary haemorrhage

When a surgeon undertakes a laparotomy for a lacerated liver he can by no means at his disposal assess the true amount of liver damage, for a small surface laceration may be accompanied by the most severe intrahepatic damage. He is therefore in no position to offer a prognosis as to the outcome of the operation, no matter how successful he may have been in suturing the damaged viscus.

Post-operative jaundice occurred in five cases in the present series and usually within the first week of convalescence. In part this is probably related to cellular damage, but all these cases had previously

been given blood and it is impossible to exclude the possibility of a transfusion reaction.

DAMAGE TO THE BILIARY RADICALS When the liver is lacerated, as well as there being vascular and sinusoidal damage, fine biliary radicals are divided and bile in addition to blood pours into the peritoneal cavity. Should surgical intervention be successful in controlling haemorrhage, but fail to ensure either that no further bile flows into the peritoneal cavity or that adequate drainage for further uncontrolled biliary secretion is provided, then the majority of those intra-abdominal complications which arise in the post-operative period can be attributed to the peritoneal irritation consequent upon the biliary outflow.

In this particular series the following complications were observed: Peritonitis (4 cases), ileus (3 cases), intestinal obstruction (3 cases), residual abscess (1 case), and burst abdominal wound (2 cases).

'HEPATO-RENAL SYNDROME'

This term 'hepato-renal syndrome' is an uncertain entity and has been applied to all sorts of conditions in which terminally there is a progressive renal failure of unexplained origin. In cases of hepatic trauma it has been postulated that a nephrotoxin is elaborated by the damaged liver and on this account it was suggested by Boyce (1941) and later by Gans (1955) that this was the most important reason that 'the removal of devitalized liver tissue is indicated'. This hypothesis cannot go unchallenged and the following points summarize the arguments that can be advanced against it.

(1) Experimental injury to the liver in dogs, rats, and rabbits has been unsuccessful in producing the type of injury found in humans (Orr and Helwig, 1939; Helwig and Orr, 1932). (2) There are many cases of survival described in which (*a*) massive liver damage was sustained, (*b*) where liver sequestra have been either removed surgically or discharged spontaneously from a drainage hole a considerable period after the initial injury (Sanders, Macguire, and Moore, 1949; Clarke, 1941; Pilcher, 1942; Thomason, 1952; Cohn, 1947). In none were there any associated abnormal urinary symptoms. (3) Finally, one may therefore ask, 'If damaged liver can produce a nephrotoxin why does it not occur in every case'?

Whereas the mechanism of acute post-traumatic renal failure is not yet fully explained and is probably related to the extent and duration of the traumatic hypotension, it seems most unlikely that a specific nephrotoxin is elaborated by the liver. Incompatible blood transfusion, crushing, shock, and many other complex factors are involved, each of which may play a part in producing this syndrome. In only one of the cases under review did post-operative anuria occur.

CLINICAL FEATURES AND DIAGNOSIS

When a patient sustains an injury to the liver, he may be killed immediately or die before admission to hospital; he may survive long enough to be admitted to hospital (the common type), or he may present days or even weeks later with delayed manifestations (*e.g.*, abdominal mass, delayed rupture, liver or subphrenic abscess, haemobilia, and so on). It is with the common type that we are concerned and these patients present with varying degrees of shock and abdominal pain.

In this particular series shock was present in varying degree and some patients presented the typical appearance of blood loss. They were pale and sweating with sometimes a tinge of cyanosis of the lips and in others air hunger. One would expect that the degree of shock would bear a direct relationship to the extent of the laceration but this was not so. In one of the cases an elderly man involved in a car accident, who had a blood pressure of 120/90 mm. Hg and a pulse rate of 60 when admitted to hospital, was found at operation to have an extensive inverted Y laceration of the right lobe of the liver. Conversely, a small boy of 11 years who had sustained a 1 in. laceration of the liver, was profoundly shocked on admission. In a more recent review of 21 cases of liver injury, 47·5% of cases when first seen had systolic blood pressures of over 100 mm. Hg. It is apparent that neither the general appearance of the patient nor the blood pressure readings can be regarded as accurately reflecting the extent of blood loss or of hepatic damage. It would be most unwise to allow either of these uncertain features to affect surgical judgment when it comes to a question of whether or not to explore the abdomen, for the physiological response to blood loss varies considerably from individual to individual.

The abdominal physical signs found in these cases are considerably modified by the presence of associated intra-abdominal injuries. In this series of 30 confirmed cases only 50% were uncomplicated, and in the remainder the associated organs injured all lay in the upper abdomen close to the liver (stomach 3, jejunum 1, transverse colon 1, omentum and mesentery 4, spleen 3, kidney 2, gall bladder 1, pancreas 1, adrenal 1). The majority, therefore, complained of generalized abdominal tenderness most marked perhaps in the upper abdomen or epigastrium. In only six was there pain, tenderness, and rigidity related to the right hypochondrium. On the

other hand, in an unpublished review of another 21 cases of uncomplicated liver injury, a presumptive pre-operative diagnosis was possible in 85% of cases, but even so in half of these the main complaint was of generalized abdominal tenderness. On more than one occasion the physical signs were completely misleading for in one case pain was most marked in the left iliac fossa.

OVERALL MORTALITY RATES AND TRENDS

The mortality rate in subcutaneous liver injury is extremely high. In the present series of 30 confirmed cases, 18 died and 12 survived, giving a mortality rate of 60%. On the other hand, taking the series as a whole, there was a steady decrease in the mortality rate, which coincided with the establishment of a regional blood transfusion service and with the increasing use of whole blood at the earliest possible time (Fig. 3). This overall mortality rate does not compare unfavourably with others found in the literature (Tilton 62·5%; Vance 100%; Lamb 68%; Wright *et al.* 81·3%; Allen 66·6%). As would be expected, as the number of associated injuries increased, so did the mortality rate. In the present series Fig. 4 demonstrates this point.

It is commonly held that the mortality rate of closed liver injuries is higher than in penetrating ones. No comparison was possible in the present review for only one case of open injury occurred. This view is based on four critical reviews by Edler (1887), Tilton (1905), Boljarski (1910), and Cohn (1947), each of whom demonstrated that closed liver injuries were associated with about a 40% higher mortality rate than with open injuries.

CAUSES OF DEATH

Of the 18 deaths in this series, there was evidence that in 11 cases no blood transfusions were undertaken or that it was either in inadequate amounts or too late. In fairness it must be stated that these deaths occured mainly in the years between 1926 and 1936 when a regional blood transfusion service was not in existence. Of the remaining seven, three died of complications attributable to uncontrolled biliary leak from the damaged liver. Two developed burst abdominal wounds at 11 and nine days respectively and both died at re-operation for suturing the wound. One collapsed suddenly for no apparent cause, and the other vomited and aspirated the stomach contents into the trachea. The third case developed intestinal obstruction. One patient died on the sixth day of anuria and renal failure; one patient developed a secondary haemorrhage on the ninth day, and one patient survived 56 days, but finally died from associated pulmonary tuberculosis. Finally, one child died shortly after operation and at necropsy the only definite finding was that histologically there was considerable diffuse intrahepatic haemorrhage.

It would seem, therefore, that there are three main causes of death: 1, Inadequate pre-operative preparation, 2, inadequate control of haemorrhage and biliary leak at operation, and 3, there are some cases in which the intrahepatic damage is so diffuse as to render the prognosis most doubtful. Because of this last fact the surgeon is entirely in the dark and all he can do is the minimum to control further loss of blood and bile. He can offer no prognosis and he must appreciate two things, namely, there is going to be a percentage of patients who die following

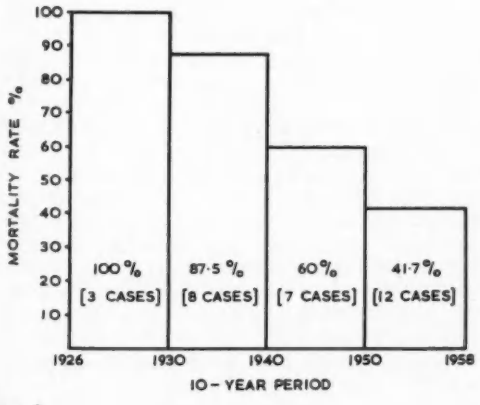


FIG. 3

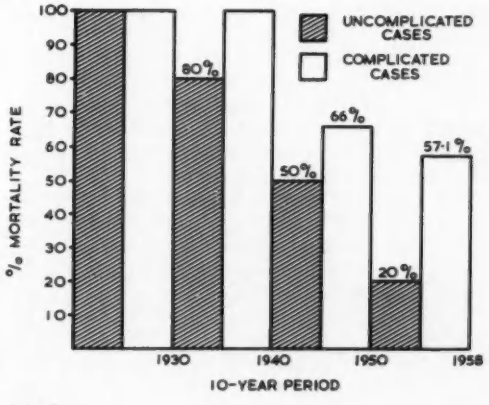


FIG. 4

successful operation, those with extensive intrahepatic injuries and there is nothing he could have done about it; there is a very small percentage of cases in which complications attributable to intrahepatic necrosis (*e.g.*, abscess, sequestra) may occur. Again nothing can be done about these at operation and he must patiently await the outcome of his initial efforts.

MORTALITY RATE IN RELATION TO TYPE OF TREATMENT

There has been divergence in opinion as to whether or not laparotomy should be undertaken in cases of ruptured liver. The case for a conservative policy hinges on the fact that the portal venous circulation is sluggish and of low pressure and that invariably, at operation, no continuing haemorrhage of any great extent is found. Cases are bound to crop up in which no major breach in intrahepatic venous circulation has occurred, but there are other factors which need most careful consideration before a régime of early surgical intervention is condemned.

The importance of the valveless hepatic veins and their close relationship to the right side of the heart has already been discussed. It is commonly held that bleeding has ceased by the time laparotomy is undertaken. This is not wholly true. It has been my personal experience that while the lacerated area is filled with clot and there is no longer any massive bleeding, there is always a slow but constant trickle into the peritoneal cavity. These clots are friable and most easily detached, but what is most important is that they conceal tributaries of either the portal or hepatic venous systems visible to the naked eye. In three cases I have seen such clots separate following the most gentle manipulations, and this was followed by a pulseless gush of blood from vessels as large as an adult-sized radical artery. With such experience it is difficult to be convinced that conservatism is either justifiable or a sound surgical policy.

Precise diagnosis is frequently impossible. Concomitant injury of liver and spleen manifests itself in exactly the same fashion as if only one was injured, namely, as intra-abdominal haemorrhage. Furthermore, in 50% of the cases in this series there was an associated injury found at operation.

No recognition has been taken of the fact that if the injury is not treated there is a continuous free flow of bile into the peritoneal cavity, with its own particular risks and consequences. This view is supported by Halberstam, Bloom, and Graham (1947) and Newell (1948), who state that laparotomy is indicated not only in every case of liver injury, but also when such an injury is merely probable, and that 'traumatic rupture of the liver is a dangerous injury. All patients with massive haemorrhage die, usually before surgery can be attempted. All those with acute haemorrhage die, if they are not given the benefit of surgery'. Statistically, three reviews support this opinion.

TABLE III

Author	*Cases Operated*		*Cases Not Operated*	
	No.	*Died (% Mortality)*	*No.*	*Died (% Mortality)*
Allen (1931)	22	14 (63·7)	2	2 (100)
Wright *et al.* (1947)	9	5 (55·5)	17	17 (100)
Mikal and Papen (1950)	7	— (38·9)	18	— (81·8)

DIFFERENTIAL DIAGNOSIS

This, of course, involves the whole problem of diagnosis of abdominal injuries. In interpreting the physical signs, four factors must be considered: the actual injury to the abdominal wall, the low-grade peritoneal irritation produced by a haemoperitoneum, the more severe type resulting from actual infection of the peritoneal cavity by intestinal contents, bile, or urine, and finally the amount of blood lost.

The localizing physical signs, *e.g.*, the marked tenderness in the right hypochondrium, are probably determined by the site at which the external force strikes the abdominal wall, but which viscus is injured is determined by the direction of this external force. This can be the only explanation for those cases of liver injury in which the physical signs are most marked in a quadrant other than the right hypochondrium, *e.g.*, the left iliac fossa. Haemoperitoneum is productive only of a low-grade peritoneal irritation and the physical signs are dependent entirely upon its extent and its duration. Furthermore, the injury to the abdominal wall may have been diffuse and the differentiation between guarding and rigidity is then difficult.

All that can be said with confidence is that the patient is suffering from an intra-abdominal haemorrhage as the variations in local physical signs are so great. The adoption of a purely academic outlook in the diagnosis and treatment of abdominal injuries, and to say, this is a case of ruptured liver, or this is a case of ruptured spleen and so on, is wrong. In clinical practice we are presented with a patient with an abdominal injury and it is impossible to sort out the clinical features and relate them to one or other organ. There are only two decisions which are required: Whether or not surgery is to be undertaken, and if so, when?

Two things only must be considered: 1 Has the patient lost blood into the peritoneal cavity and is he continuing to lose blood? 2 Are there early signs

of peritonitis and is it spreading? In so far as those cases are concerned which are admitted with profound shock and blood loss, the decision is obvious, but the difficulty arises where the degree of haemorrhage or peritoneal irritation is minimal. The signs of haemoperitoneum are slight, productive only of mild generalized tenderness with slight guarding or diminution in bowel sounds, and the compensatory ability of some patients is so great that up to 3 pints of blood can be lost without much alteration in blood pressure and pulse rate. Even if these minimal signs are present and a liver injury is suspected, for the foregoing reasons, because the patient is being operated on when his general condition is relatively good, early surgery is to be recommended.

If for some reason or another a decision is made to observe the patient, then a rising pulse rate is an earlier and more accurate assessment of blood loss than change in blood pressure. Similarly as a reflection of the development of peritonitis of any degree, alteration in the quality, or absence of bowel sounds, is the most important physical sign.

The presence of haematuria or multiple fractured ribs must not be allowed to deter a surgeon from operating. This is a great diagnostic pitfall and if the abdominal physical signs leave the slightest possible doubt that some intra-abdominal injury has taken place, then laparotomy should be performed. Considerable judgment is required to decide whether operation is needed or not, but any deterioration in the general or local condition of the patient warrants the most serious consideration of laparotomy. There is no disgrace in operating and finding nothing.

TREATMENT

From what has been said in the previous sections and if the policy of early surgical intervention is accepted, then treatment must be directed towards early and adequate replacement of the blood lost, early control of bleeding, and at the same time control of biliary outflow or the provision of adequate external drainage from the lacerated area. No further comment will be made on the question of transfusion, only to repeat that in 11 of the 18 deaths in this series death was attributable either to delay or inadequacy of blood replacement. When operation is undertaken the whole abdominal cavity must be systematically examined to exclude any other injury.

There remain to be discussed details of surgical technique and it is around this point that there has probably been the greatest controversy of all. There is one important feature which must be emphasized above all else. If hepatic surgery is to be undertaken, a needle long enough to be passed through the whole thickness of the liver must be available. A Kousnetzoff needle is not an instrument available in all theatres, whereas a 9 in. silver malleable probe with an eye will prove more valuable than the largest available needle of the ordinary variety.

SURGICAL APPROACH Grey Turner's (1940) advice must always be borne in mind: 'The patient will not die from a very big incision, but may very likely succumb if some important injury is overlooked . . .'. In this respect the question arises whether or not there is justification under certain circumstances in converting a simple upper abdominal incision into a thoraco-abdominal one.

The crux of the matter seems to be, Is it going to be of any value? If on palpation a deep cleft which is bleeding severely can be demonstrated, and it is felt that this would be amenable to some form of suturing, then there can be no reason for not doing this, for suturing is preferable to packing. On the other hand, if a stellate or grossly comminuted injury is felt, in which suturing would be out of the question, then there is no point in doing it and packing should be resorted to. The additional risks of a thoraco-abdominal incision must be weighed against the risks of closing the abdomen with bleeding incompletely controlled.

TREATMENT OF THE LIVER WOUND There can be no uniform method applicable to all varieties of liver injury. These wounds vary in size and depth, and may be simple surface breaches of Glisson's capsule, full-thickness divisions, or stellate and comminuted injuries with variable degrees of separation. The specific measures which must be considered are debridement, packing, suturing, drainage, or a combination of all.

Debridement That there is a place for the removal of almost completely or completely detached segments of liver, whose viability is in doubt, cannot be denied, for they are potentially sources of infection. The fulfilment of this ideal may be difficult, for there is not only the worry of having to accomplish haemostasis from a newly created raw surface, but there is the worry of extending the surgical procedure in a patient who is already critically ill. In this series what liver was removed was either free, small, or fragmented. In the case in which deep fissures are present which extend through most of the liver substance, the additional risks of removal may well be preferable to re-attaching them, so running the risks of repeated secondary haemorrhages which are even more difficult to control and may necessitate the use of a thoraco-abdominal approach. This view is supported by the experience of Sanders *et al.*, who, in two cases in which a deep laceration of the lateral third of the right hepatic lobe was present, had to operate a second time, using a thoraco-abdominal

approach to control secondary haemorrhage from the necrosed fragments of liver which had been re-attached to the main bulk of the liver at the first operation. There seems to be one exception to this, namely, deep lacerations immediately to the left of the falciform ligament where the whole of the left lobe of the liver has been detached. This is the site at which the portal veins and hepatic vessels bifurcate and where there is a probability that the detached fragment has retained its blood supply. The porta hepatis should be carefully examined to determine this point before suturing is undertaken. Such a procedure was performed with success in two cases in this series.

Packing Packing is one of the traditional methods of controlling haemorrhage in liver injuries. Three substances have been used, namely, gauze, local tissues (falciform ligament, omentum, muscle), and synthetic coagulants.

Gauze packing was associated with the highest mortality rate in this series. Of the eight patients in whom it was used, five died. If packing is to be resorted to, it must be placed in a position in which it can exert pressure and not be easily dislodged. The only part of the liver in which this can occur is the dome, where the space between liver and diaphragm is so small that it can exert effective pressure and remain where it has been placed. On the undersurface packing moves every time the patient breathes and it soon becomes dislodged and ineffective. If it is used in such a situation it then becomes nothing more than a gesture. The use of packing is therefore indicated in cases of comminution of the liver not amenable to suturing, in the dome, or posterior surface, and it is obviously of value in those patients whose general condition is so critical that the operation must be abandoned with haste. Quite apart from this, other theoretical objections have been raised against packing. 1 It must be tight or it is of no use: if too tight it will produce pressure necrosis and favour secondary haemorrhage. 2 It tends to dam any flow of bile and is a potential source of infection leading to localized pockets of bile, abscesses, hepatitis, subphrenic infection, and increases the risk of chest complications (Madding, 1955).

Local tissues have been used in an effort to avoid a gauze pack. However, they are often difficult to reflect into position and if they are to be effective need to be used as free grafts. Synthetic coagulants are useful in the control of superficial laceration either alone or in combination with suturing. Their use, however, in large amounts or in deep lacerations in combination with suturing must be regarded with some caution for their rate of absorption is slow and may exceed the period required for healing; they are not always absorbed and may lead to their own quota of post-operative complications. These have included subphrenic abscess, hepatic abscesses, intestinal obstruction from adhesions, and the erosion of the oxidized cellulose through the intestinal wall.

Suturing This is undoubtedly the most effective means of both controlling haemorrhage and biliary drainage, and should be employed whenever possible. Two types of injury seem to lend themselves to this form of treatment: the linear full-thickness and partial-thickness injuries. Preliminary occlusion of the vessels lying in the free edge of the lesser omentum will help to reduce the amount of bleeding from the raw liver surface, but not completely, for that does not control any back flow from the hepatic veins. Before suturing, attempts should be made to identify and ligate any major vascular radicals visible on the surface, and when such suturing is finally undertaken it should be not merely across a wound, for such stitches have no effect whatsoever in controlling either biliary outflow or further haemorrhages. The important sutures are those which run parallel with the wound edges, for it is these which compress the vascular and biliary channels. If sutures are placed parallel with the torn surfaces and effect haemostasis, then the liver substance lying between those sutures and the tear is liable to undergo necrosis. It is difficult to see any alternative and the risk of possible necrosis must be accepted as the price to be paid for effective haemostasis. (On several occasions I have placed such sutures $\frac{1}{2}$ in. from the tear and have found that not only do the sutures hold well, but that no complication which has been mentioned has occurred.) When considerable fissuring is present, attempts at suturing are not always successful, but even so, considerable patching of these wounds can sometimes be accomplished. When resection of devitalized tissue has been undertaken, suturing is of course essential in an attempt to control bleeding from the torn surface.

One last consideration is that it has been shown that the tight closure of these lacerations may lead to certain complications. A closed cavity may be created within which blood, bile, or pus may accumulate, leading to cyst or abscess formation or the syndrome of traumatic haemobilia. On this account Kreig (1936), Hawthorne, Oaks, and Neese (1941), and Sparkman (1953) suggested that a small drain should be inserted into the depths of a sutured hepatic laceration.

Drainage Although drainage was not undertaken in many cases in this series, without adverse complications, it cannot be regarded as a good surgical practice. Omission to drain can only be attributed to failure to appreciate the fundamental pathology or to the supreme confidence of the

operator that further biliary leak has been prevented. The biliary radicals are small, difficult to detect even at necropsy, and neither the appearances nor the size of the wound can give any indication of the possible amount of further biliary flow. In addition, there is always the question of possible necrosis. The placing of drains, not only in appropriate positions on the surface of the liver, but also when the occasion demands, in the liver substance itself, must be regarded as being one of the most important features in the management of liver injuries.

SUMMARY AND CONCLUSIONS

Clinical research which involves not only the collection and analysis of a given number of cases, but also the comparison of results obtained with those in other published series, is always beset with difficulties. Apart from the question of gathering in these cases, it is invariably found that there are gaps in the recording of the details regarded as being essential in the preparation of any statistical analysis. So it has been with this series. It has been impossible to produce figures of such accuracy that they would withstand a truly scientific criticism. Quite apart from this, there are 'clinical' features such as the individual patient's reaction to blood loss, and surgical and anaesthetic skill, which are so varied that it is impossible to translate them into terms which can be expressed or recorded as graphs or figures. One is left only with impressions and individual opinions.

In the introductory paragraph of this paper certain questions were posed, and, bearing in mind what has been said, the conclusions arrived at are as follows:

Liver injuries are uncommon. They occur in between 1 in 1,300 and 1 in 4,000 cases admitted to hospital. Hepatic injury occurs if not with equal, then with slightly greater frequency than splenic injury, but which is injured when a patient is involved in an accident is a matter of chance, for both these organs lie closely together in the upper abdomen and whether one or the other or both are damaged depends on the site, direction, and extent of injury. Attention is drawn to the difficulties of precise diagnosis and that in these cases multiple intra-abdominal injuries may often be sustained.

The greatest single cause of death is haemorrhage and shock, but late complications following operation can be attributed mainly to a continued biliary leak from the torn liver surface.

The mortality rate is still high in closed liver injuries. In this series the overall mortality rate is 60% and this compares favourably with figures obtained in other series.

The one pathological feature not always appreciated is that the surface laceration is only part of varying degrees of generalized liver damage, and that although the regenerative powers of the liver are excellent, on occasions the intrahepatic damage may be so extensive that death occurs shortly after an apparently successful operation.

There is no one correct treatment, especially where the actual technique of hepatic haemostasis is concerned. Packing if possible should be avoided, but if it has to be resorted to, it must be placed in a position where not only will it remain but where it will exert pressure. The only place where this can occur is over the dome and posterior surface. Suturing is to be preferred and should be used whenever possible.

There is a place for more radical surgery on a few occasions. Where a segment of liver is almost completely detached it should be removed, and where a freely bleeding laceration at the dome or posterior surface is detected and it is felt to be amenable to suturing, then there is justification for converting a simple laparotomy into a thoraco-abdominal exploration to ensure good vision for effective haemostasis.

The most important single factor which can reduce the overall mortality rate is the early and adequate transfusion of whole blood. Early operation is considered advisable, and although on a few occasions resection of hepatic tissue may be necessary, in general the simpler the technique used to control both haemorrhage and biliary effusion, the better. Drainage of the peritoneal cavity is considered to be not only good surgical practice, but essential in cases of liver injury.

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REFERENCES

Allen, A. W. (1931). Internal injuries without penetrating wounds. *New Engl. J. Med.*, **205**, 34-38.

Boljarski, N. (1910). Ueber Leberverletzungen in Klinischer und experimenteller Hinsicht, unter besonderer Berücksichtigung der isolirten Netzplastik. *Arch. klin. Chir.*, **93**, 507-547. (Cited by Thorlakson and Hay.)

Boyce, F. F. (1941). *The Rôle of the Liver in Surgery*. Charles C. Thomas, Springfield, Illinois.

Clarke, R. (1941). A case of liver 'sequestrum' complicating subcutaneous rupture of the liver. *Brit. J. Surg.*, **28**, 544-548.

——(1954). Closed abdominal injuries. *Lancet*, **2**, 877-885.

Cohn, R. (1947). The treatment of traumatic injuries of the liver. *Stanf. med. Bull.*, **5**, 120-124.
Edler, L. (1887). Die traumatischen Verletzungen der parenchymatösen Unterleibsorgane. *Arch. klin. Chir.*, **34**, 343-409. (Cited by Tilton, B. J.)
Gans, H. (1955). *The Anatomy of the Intrahepatic Structures and its Repercussions on Surgery*. Elsevier Publishing Co., Amsterdam and New York.
Grey Turner, G. (1940). Some notes on abdominal injuries. *Brit. med. J.*, **1**, 679-682.
Halberstam, C., Bloom, J., and Graham, A. (1947). Laceration of the liver. *Industr. Med.*, **16**, 52-57.
Hawthorne, H. R., Oaks, W. W., and Neese, P. H. (1941). Liver injuries with a case report of repeated hemorrhages through the biliary ducts. *Surgery*, **9**, 358-460.
Helwig, F. C., and Orr, T. G. (1932). Traumatic necrosis of the liver with extensive retention of creatinine and high grade nephrosis. *Arch. Surg.* (*Chicago*), **24**, 136-144.
Hinton, J. W. (1929). Injuries to the abdominal viscera. *Ann. Surg.*, **90**, 351-356.
Kreig, E. G. (1936). Hepatic trauma. Analysis of 60 cases. *Arch. Surg.* (*Chicago*), **32**, 907-914.
Lamb, C. A. (1939). Rupture of the liver. *New Engl. Med. J.*, **221**, 855-859.
Lewis, D., and Trimble, I. R. (1933). Subcutaneous injuries of the abdomen. *Ann. Surg.*, **98**, 685-697.
Madding, G. F. (1955). Injuries of the liver. *A.M.A. Arch. Surg.*, **70**, 748-756.
Mikal, S., and Papen, G. W. (1950). Morbidity and mortality in ruptured liver. *Surgery*, **27**, 520-525.
Musselman, M. M., Glas, W. W., and Campbell, D. A. (1955). Hepatic injuries. *Amer. J. Surg.*, **89**, 748-752.
Newell, C. E. (1948). Traumatic rupture of the gall bladder and liver. Report of a case with generalised bile peritonitis. *Ibid.*, **76**, 466-471.
O'Neill, J. N. (1941). Traumatic rupture of the liver. *Calif. west Med.*, **54**, 68-70.
Orr, T. G., and Helwig, F. C. (1939). Liver trauma and hepatorenal syndrome. *Ann. Surg.*, **110**, 682-692.
Papen, G. W., and Mikal, S. (1950). Liver trauma. *Rev. Gastroent.*, **17**, 633-642.
Pilcher, L. S. (1942). Massive rupture of the liver. *Ann. Surg.*, **116**, 827-832.
Sanders, G. B., Macguire, C. H., and Moore, R. H. (1949). Massive rupture of the liver. *Amer. J. Surg.*, **78**, 699-705.
Sparkman, R. S. (1953). Massive hemobilia following traumatic rupture of the liver. *Ann. Surg.*, **138**, 899.
Thomason, T. H. (1952). Massive rupture of the liver. *Texas J. Med.*, **48**, 824-826.
Thorlakson, P. H. T., and Hay, A. W. S. (1929). Rupture of the liver. *Canad. med. Ass. J.*, **20**, 593-598.
Tilton, B. T. (1905). Some considerations regarding wounds of the liver. *Ann. Surg.*, **61**, 20-30.
Vance, B. M. (1928). Subcutaneous injuries of the abdominal viscera. Anatomic and clinic characteristics. *Arch. Surg.* (*Chicago*), **16**, 631-679.
Wright, L. T., Prigot, A., and Hill, L. M. (1947). Traumatic rupture of the liver without penetrating wounds. A study of 32 cases. *Ibid.*, **54**, 613-632.

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Methods and techniques

The microvilli of the small intestinal surface epithelium in coeliac disease and in idiopathic steatorrhoea

MARGOT SHINER AND M. S. C. BIRBECK

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SYNOPSIS This paper records the electron microscopic appearances in patients with coeliac disease and idiopathic steatorrhoea, and draws attention to the changes in the microvilli.

The existence of rod-shaped extensions of the free surface of the villous cells, forming the brush border, has been known since 1857 when Brettauer and Steinach first reported their studies in rabbits, guinea-pigs, dogs, and man. This was confirmed by Granger and Baker (1949) in the first electron microscope investigation of the intestinal epithelium of rats. They demonstrated the brush border as cylindrical perpendicular projections with rounded apices, averaging 0·62μ in length and 0·08μ in width, and estimated the number of projections as 3,000 per cell. Zetterquist (1956) found these projections, or microvilli, in fasting mice to be of variable length (average 0·91 $\pm$ 0·09μ) though more constant in width (average 0·10 $\pm$ 0·003μ). He estimated 650-700 microvilli per cell, increasing its absorbing surface by about 14 times. Palay and Karlin (1959) described the microvilli in rats as regular, evenly packed, rod-like projections whose cytoplasm is continuous with that of the main cell. Their average length was 1μ and their width 0·07μ. The estimated number of projections per cell was 1,000, increasing its absorptive surface by a factor of 24. Hartman, Butterworth, Hartman, Crosby, and Shirai (1960) reported in the human a range of microvillous height of 0·8 to 1·3μ (15 normal and four non-sprue patients) with a maximal width of 0·12μ. It is clear that one of the functions of the microvilli would be to increase the absorptive area to a considerable degree and any reduction in size or number of the microvilli would impede the efficient absorption of food.

Hartman *et al.* (1960) studied the surface epithelium with the electron microscope of a patient with non-tropical sprue or idiopathic steatorrhoea before treatment and five days after the start of a gluten-free diet. They found the brush border abnormal in both instances. In addition, they found an abnormality in a proportion of mitochondria in the apical cytoplasm of the cells, as well as some unidentified bodies and granules not present in the 'normal' cell. Zetterquist and Hendrix (1960) observed stunted microvilli to be a feature of non-tropical sprue in one patient, becoming normal after treatment with a gluten-free diet. We are here reporting on our findings of the appearances of the microvilli in seven 'control' patients, eight patients with coeliac disease, and seven patients with adult idiopathic steatorrhoea or non-tropical sprue. In the last two groups we have studied the brush border in relation to treatment with a gluten-free diet.

METHOD AND MATERIAL

All 22 patients were fasted for 12 hours and were then intubated with the peroral small intestinal biopsy tube (Shiner, 1956) and multiple specimens were obtained from the last part of the duodenum or upper jejunum. Fixation of the specimens was begun within two to five minutes after cutting off the specimens from their blood supply. The biopsies from each patient were fixed in a 10% solution of formal saline for light microscopy and in 1% osmium tetroxide buffered at *p*H 7·4 (Palade, 1952). Only those specimens showing adequately preserved surface epithelium on light microscopy were included in this study so that their appearances could be compared with those of the electron microscope. The epithelial cell heights were measured with a micrometer by light microscopy and the average of 10 cell counts per biopsy per patient was determined.

For the electron microscope, after fixation in 1% osmium tetroxide for two hours the tissue was washed briefly and then dehydrated in ethanol. Because of the large size of the specimens, they were trimmed at this stage at their periphery and small pieces of tissue, less than 0·5 mm., were embedded in araldite (Glauert, Rogers, and Glauert, 1956). Sections were cut and placed on a grid for examination on a Siemens E.M. 100 electron microscope. Some of these sections were stained with lead hydroxide on the grid. Several micrographs (×8,000) were taken of the microvilli and the epithelial cells from each patient. The sites chosen for micrography were those of the tips and sides of intestinal villi, where present. The micrographs were enlarged to 32,000 × and from the prints measurements were made of the length of the microvilli. The distance measured was taken from a line running through the tips of the microvilli to the level of the plasma membrane from which the microvilli project. Measurements of the number of microvilli per cell or unit area were not made because variation in shape of microvilli, found in the pathological specimens of our group but not in the normal ones, made it impossible to be accurate.

The appearances and measurements of the microvilli were recorded by one of the authors independently of the other author's assessment of the patient's clinical state, the overall histological appearances of the mucosal biopsies, and the measurements of surface cell heights.

Of the seven patients chosen as controls (Table I), five did not suffer from steatorrhoea, in one patient (F.T.) who was symptomless, faecal fat estimations were not done, but one patient (C.G.), with extensive ulcerative colitis probably involving the terminal ileum, had mild steatorrhoea. Histologically this group showed a normal mucosa, although in two (F.T. and G.D.) the villi appeared shorter or more irregular than normal.

The ages of the eight children with coeliac disease ranged from 12 months to 11 years 10 months. Only two patients (J.N. and V.B.) were untreated at the time of biopsy. In the other six children the duration of treatment with a gluten-free diet was correlated to the time of biopsy. All six were in clinical remission but only one (J.L.) showed no gluten sensitivity, demonstrated by continued good progress after gluten had again been introduced into the diet. Gluten sensitivity was not tested in McG.A.

Of the seven patients with idiopathic steatorrhoea, three were untreated and in clinical relapse at the time of biopsy. The other four had received a gluten-free diet from one to six months before biopsy. Two of these (M.W. and J.B.) were in remission, one (E.A.) showed no response clinically or biochemically, and one (J.J.) had been in clinical remission before it was decided to put him on the diet, though his stools, which were formed, contained an excess amount of fat.

RESULTS

LIGHT MICROSCOPY The light microscopy appearances of the specimens from the 'control' group (Fig. 1) consisted of long slender villi, covered by tall columnar epithelium (Doniach and Shiner, 1957).

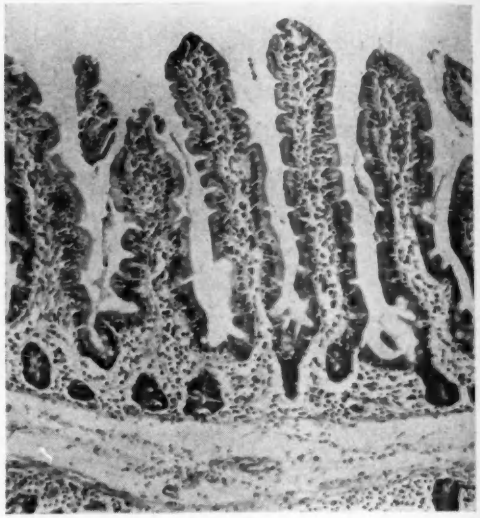

FIG. 1. *Normal mucosa of the upper jejunum. Haematoxylin and eosin × 100.*

The epithelial cell height measured on the average 35·8μ with a range of 31·0μ to 42·9μ (Table I). The cell nuclei appeared elongated, dark staining, and basally situated. The brush border was not studied with the light microscope. A few goblet cells were interspersed between the villous cells. The mucosal layer showed a thickness about one fourth of the height of the villi and contained scanty glands of Lieberkühn.

The histological appearances in the coeliac group (Shiner, 1960) showed a varied picture. In the untreated children (J.N. and V.B.) villi had almost completely disappeared and mucosal thickness had increased with hyperplasia of the glands of Lieberkühn. The surface epithelium appeared low columnar, cuboidal, or stratified, and the nuclei showed gross irregularity in size and position. The surface cell height measured less than half the normal (Fig. 2). In J.L., treated with a gluten-free diet for six years and showing no sensitivity to gluten when wheat was re-introduced, the biopsy was normal, as was the surface cell height. Of four children treated with a gluten-free diet for four to nine years but remaining sensitive to gluten, two showed a normal biopsy with normal cell height measurements, and two had subtotal villous atrophy with reduced surface cell height and irregular nuclei. In one child (McG.A.), on a gluten-free diet for only two years, sensitivity to gluten was not tested.

TABLE I

Patient Sex Age	*Diagnosis*	*Clinical State*	*Steatorrhoea*	*Treatment*	*Light Microscopy*		*Electron Microscopy*		*Comments*
					Histology	*Surface Cell Heights (μ)*	*Length of Microvilli (μ)*	*Regularity of Microvilli*	
C.B. M 36	Folic acid deficiency	Megaloblastic anaemia	Absent	Vitamin B_{12}, folic acid	Normal	32·3	1·09	Regular and tightly packed	
M.M. F 29	Diarrhoea ? cause	Diarrhoea and weight loss	Absent	G.F.D., folic acid	Normal	38·0	1·03	Regular and tightly packed	
A.H. M 46	Haemate-mesis	Iron-deficiency anaemia	Absent	Nil	Normal	31·0	1·09	Regular and tightly packed	
W.R. M 56	Haemochro-matosis	Well	Absent	Bleeding	Normal	37·6	1·09	Regular and tightly packed	
C.G. M 15	Ulcerative colitis	In relapse	Present	Prednisone	Normal	42·9	1·19	Regular and tightly packed	
G.D. M 61	Pernicious anaemia	Megaloblastic anaemia	Absent	Vitamin B_{12}	? Normal	31·7	0·75	Regular and tightly packed	Variable size of villi
F.T. M 36 (staff)		Symptom free	Not tested	—	? Normal	37·3	0·84	Variable	Variable size of villi
J.N. F 2½	Coeliac disease	In relapse	Present	Nil	S.V.A.	15·5	0·75	Irregular and loosely packed	
V.B. F 1	Coeliac disease	In relapse	Present	Nil	S.V.A.	21·1	0·90	Loosely packed	
J.L. M 10	Coeliac disease	Remission	Absent	G.F.D. for 6 years	Normal	34·3	1·03	Regular and tightly packed	No gluten sensitivity
L.C. F 12	Coeliac disease	Remission	Absent	G.F.D. for 9 years	Normal	34·0	0·87	Regular but loosely packed	Gluten sensitive
D.B. M 10½	Coeliac disease	Remission	Absent	G.F.D. for 4 years	Normal	41·9	0·84	Regular but loosely packed	Gluten sensitive
B.D. F 10	Coeliac disease	Remission	Absent	G.F.D. for 7 years	S.V.A.	21·5	0·70	Regular and tightly packed	Gluten sensitive
S.H. F 10	Coeliac disease	Remission	Absent	G.F.D. for 8 years	S.V.A.	23·1	0·62	Irregular and loosely packed	Gluten sensitive
Mc.G.A. F 10	Coeliac disease	Remission	Absent	G.F.D. for 2 years	S.V.A.	22·8	0·85	Irregular and loosely packed	Gluten sensitivity not tested
G.H. M 43	Idiopathic steatorrhoea	In relapse	Present	A.C.T.H., folic acid	S.V.A.	27·1	0·62	Regular but loosely packed	
M.M. M 30	Idiopathic steatorrhoea	In relapse	Present	Vitamin B_{12}, folic acid	S.V.A.	28·1	0·47	Regular but loosely packed	
F.D. M 62	Idiopathic steatorrhoea	In relapse	Present	Nil	S.V.A.	31·7	0·87	Regular and tightly packed	
E.A. F 75	Idiopathic steatorrhoea	In relapse	Present	Vitamin B_{12}, folic acid, G.F.D. 6 mth.	S.V.A.	27·1	0·87	Regular but loosely packed	No response to gluten-free diet
J.J. M 70	Idiopathic steatorrhoea	Partial remis-sion	Present	Vitamin B_{12}, folic acid, G.F.D. 1 mth.	S.V.A.	24·8	0·78	Regular and tightly packed	Uncertain response to gluten-free diet
M.W. F 65	Idiopathic steatorrhoea	Remission	Absent	G.F.D. 6 mth.	P.V.A.	35·0	0·85	Regular and tightly packed	Pre-treat-ment biopsy showed S.V.A.
J.B. F 34	Idiopathic steatorrhoea	Remission	Not repeated	G.F.D. 2 mth.	S.V.A.	29·4	0·80	Irregular and loosely packed	Good pro-gress on G.F.D.

G.F.D. = gluten-free diet
S.V.A. = subtotal villous atrophy
P.V.A. = partial villous atrophy
idiopath. steatorrh. = idiopathic steatorrhoea

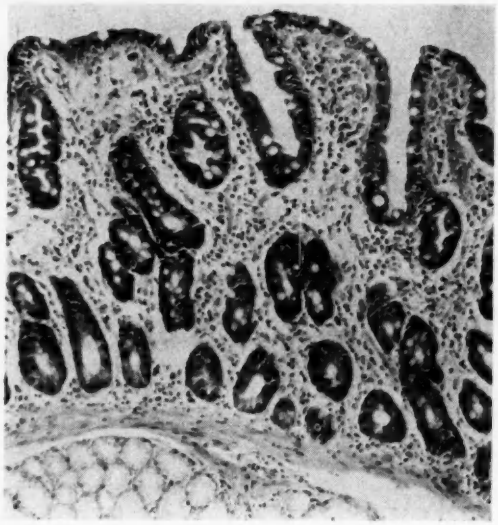

FIG. 2. *Subtotal villous atrophy in coeliac disease. Haematoxylin and eosin × 100.*

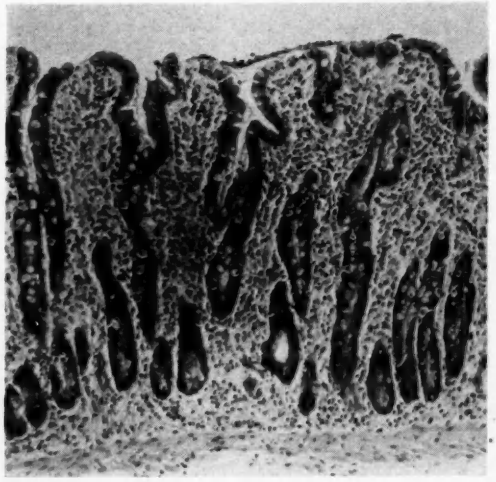

FIG. 3. *Subtotcl villous atrophy in idiopathic steatorrhoea. Haematoxylin and eosin × 100.*

In all three patients with idiopathic steatorrhoea untreated with a gluten-free diet, the biopsies showed subtotal villous atrophy (Fig. 3), reduction of surface cell height, and irregularity of the nuclei (Shiner and Doniach, 1960). The biopsies of two of the four patients with idiopathic steatorrhoea treated with a gluten-free diet for one to six months but showing no clinical or biochemical response during that time or after, had the same histopathological appearances as the untreated group. In the biopsies of the remaining two patients on a gluten-free diet with good clinical response, one (M.W.) showed a partial villous atrophy where a pre-treatment biopsy had shown subtotal villous atrophy. The surface epithelium showed improvement in height and nuclear regularity over the pre-treatment biopsy and the surface cell height was within normal limits. The other (J.B.) showed a subtotal villous atrophy in spite of the patient's improved clinical state. The average surface cell height of all biopsies in the coeliac-idiopathic group was 27·8μ.

ELECTRON MICROSCOPY Electron micrographs of the seven control patients showed normal cytological structure of the villous epithelium. In the biopsies of five patients the microvilli had a length of 1·03 to 1·19μ and were regular in size and shape as well as tightly packed (Fig. 4). The other two (F.T. and G.D.), however, had a microvillous length of 0·75μ and 0·84μ respectively although the regularity in spacing of microvilli was considered normal (Fig. 5). These two sections also showed shortening and irregularity of villi under the light microscope, and one an unusual variation in length of microvilli, from 0·78μ to 0·94μ. In neither of these two sections was there any evidence of other abnormal structures and the nuclei, mitochondria, golgi region, and cell membranes of the epithelium appeared normal.

The appearances of the microvilli in the biopsies of eight coeliac children were variable. In only one child (J.L.) were they of normal length and this correlated well with the patient's complete clinical remission, absence of sensitivity to gluten, and normal appearances of the mucosa on light microscopy. The biopsies of the remaining seven patients had shortened microvilli, measuring 0·75, 0·70, 0·90, 0·62, and 0·85μ respectively in those which also demonstrated subtotal villous atrophy on light microscopy, and 0·87μ and 0·84μ in those with normal mucosal appearances (Fig. 6). Variation in the packing of microvilli was seen in all but one biopsy. In one instance (patient S.H.) dense granules were seen in the cytoplasm of the surface cells and these were present also in one case with idiopathic steatorrhoea.

FIG. 4. *Electron micrograph from control case C.B. showing normal microvilli and upper part of small intestinal surface cell* × *32,000.*

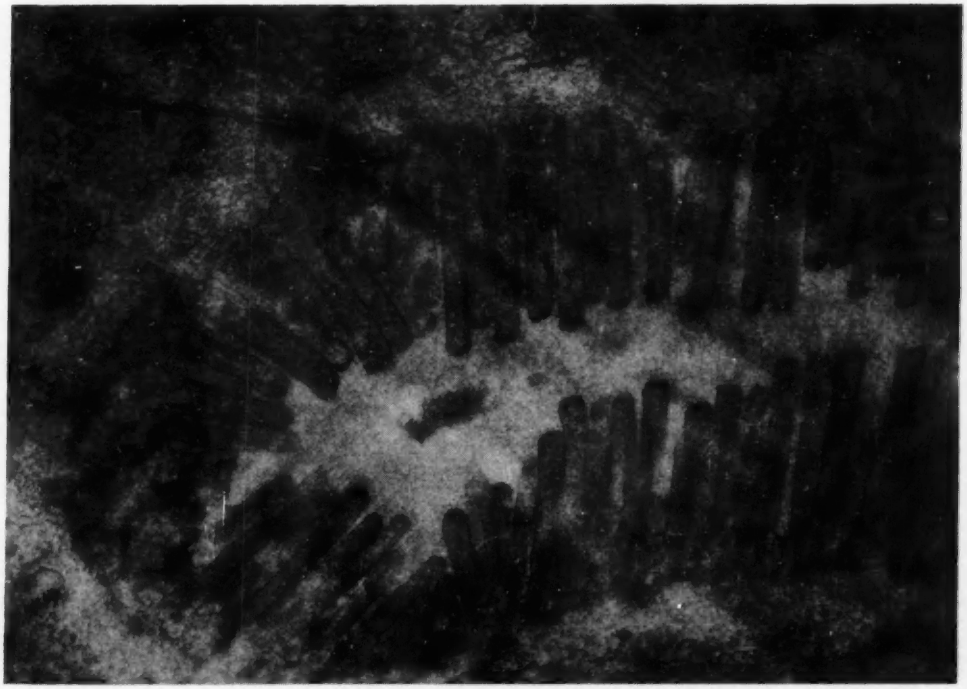

FIG. 5. *Electron micrograph from control case F.T. showing short, though regular microvilli with variation in length* × *32,000.*

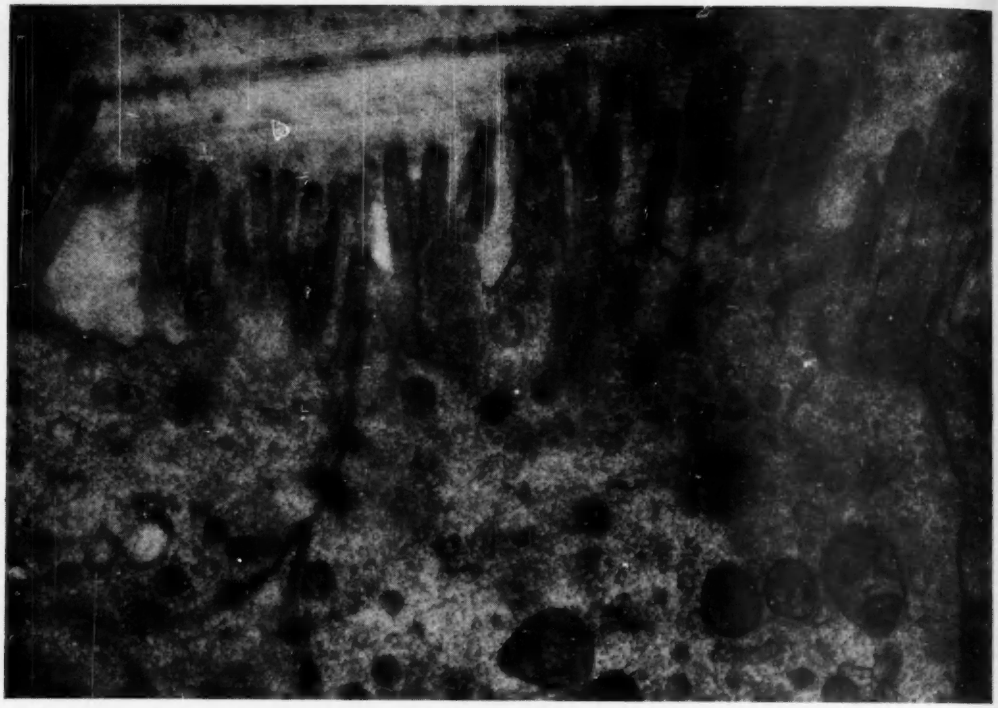

FIG. 6. *Electron micrograph from coeliac patient J.N. showing short, irregular and loosely packed microvilli* × *32,000.*

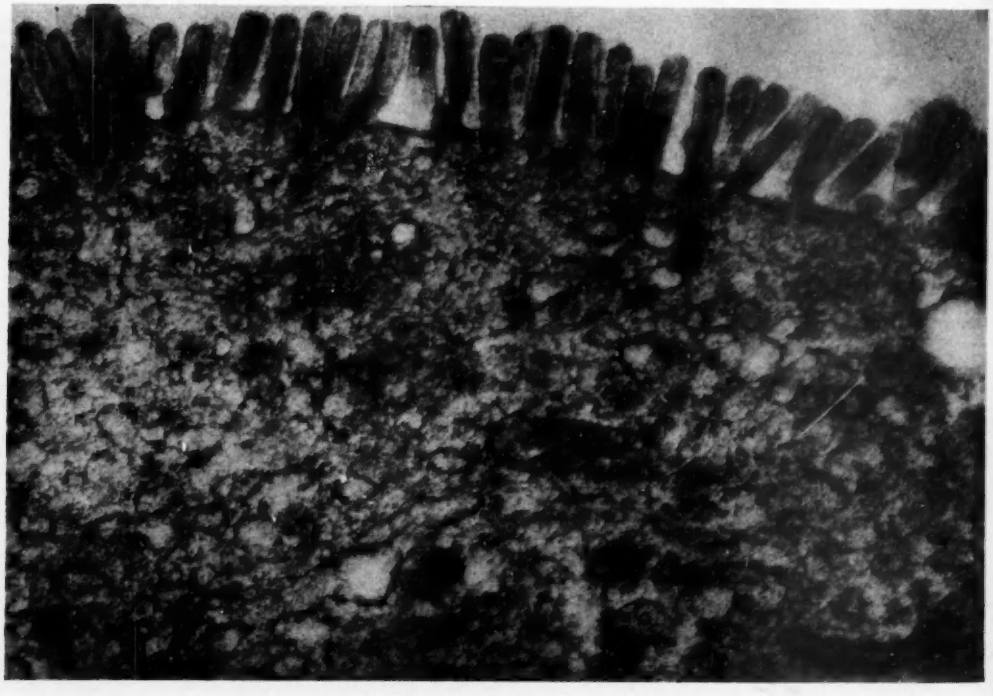

FIG. 7. *Electron micrograph from patient M.M. with idiopathic steatorrhoea showing greatly shortened and slightly wider microvilli which are regular and loosely packed* × *32,000.*

In biopsies from all seven patients with idiopathic steatorrhoea the length of microvilli was reduced, varying from 0·47μ to 0·87μ. The shortest microvilli were found in those of the untreated patients, in relapse at the time of biopsy. In the biopsy from one treated patient (M.W.) with partial villous atrophy the microvilli remained shorter than normal (0·85μ), despite the histological improvement over a previous pre-treatment biopsy. Sections of four out of the seven patients showed irregularity in size of microvilli and density of their spacing (Fig. 7).

As in the coeliac group, the cytoplasmic appearances in idiopathic steatorrhoea were, on the whole, normal. Peculiar granules and somewhat pathological nuclei could be demonstrated in one of three untreated patients (G.H.) and very abnormal cytoplasm and nuclei in a second (M.M.). These two sections also showed the shortest microvilli of this group, 0·62μ and 0·47μ respectively.

DISCUSSION

The mucosa in idiopathic steatorrhoea and untreated coeliac disease shows almost invariably a villous atrophy, which is usually subtotal but sometimes partial (Shiner and Doniach, 1960). Repeat mucosal biopsies in idiopathic steatorrhoea after gluten-free diets have shown improvement in some patients investigated by one of us; broad, though stunted villi have reappeared and taller surface cell epithelium with more regular nuclei. But complete mucosal normality has never been seen. In untreated coeliac disease the degree of villous atrophy is usually more severe than in the adult untreated or treated idiopathic steatorrhoea, but first biopsies in patients treated with a gluten-free diet over a long period of time have shown a more variable histological picture, ranging from subtotal villous atrophy to normal (Shiner, 1960). These appearances could not be correlated with the clinical state of the child. Repeat biopsies in one coeliac patient on a gluten-free diet over a period of three years showed considerable histological improvement though the overall appearances remained abnormal. Reports from other centres (Rubin, Brandborg, Phelps, Taylor, Murray, Stemler, Howrie, and Volwiler, 1960; Anderson, 1960) have indicated a possible return to histological normality after treatment with a gluten-free diet in coeliac disease.

Despite the abnormal light microscopy appearances of the surface cells and their nuclei in both coeliac disease and idiopathic steatorrhoea, the structural abnormalities of these cells under the electron microscope were less striking. The cell membrane, cytoplasmic inclusions, mitochondria, and Golgi apparatus appeared normal in 12 of the 15 patients with coeliac disease or idiopathic steatorrhea. However, the nuclei varied somewhat from the normal oval shape in being rounder, more irregular in outline, and often indented. The most striking abnormalities, however, appeared to be in the microvilli, where a reduction in height of these could be demonstrated in sections from 14 out of 15 patients with coeliac disease and idiopathic steatorrhoea and considerable irregularity and looseness of packing of microvilli in eight out of the 15 patients' biopsies.

Considering the coeliac disease-idiopathic steatorrhoea groups together, it has been shown (Table I) that the shortest microvilli were found in the untreated cases, which also exhibited a significant degree of irregularity. Amongst the treated patients only one out of 10 showed normal microvilli. The other nine had persistent abnormalities of microvilli, irrespective of the histological appearances, which ranged from normal to subtotal villous atrophy.

In the past few years much discussion has centred around the morphological cause of villous atrophy. It is known that the cells of the glands of Lieberkühn, which show active mitosis, produce the columnar cells of the villi, in which mitosis is never seen. The cells at the tip of the villi are therefore older than those at the crypt and are eventually extruded into the lumen. An absence or stunting of the villi could be due to decreased viability of the surface cells or diminished production of these. We have not found any evidence of increased destruction or disintegration of surface cells in coeliac disease or idiopathic steatorrhoea which would lead us to postulate decreased viability of these cells. With the light microscope it is common to observe a disintegrated cell being extruded at the tip of a normal villus, but in the villous atrophy of coeliac disease or idiopathic steatorrhoea this is not so evident. There is evidence only of shortening of microvilli, some alteration in nuclear shape, and shortening of the overall cell height. Diminished production of surface cells as a cause of villous atrophy is the more likely explanation, coupled perhaps with an abnormal production of cells which show a reversion from the highly differentiated columnar type to the more undifferentiated cuboidal or even stratified types. This occurs despite an apparent hypertophy of the glandular cells which show an increase rather than a decrease in mitotic figures (Shiner and Doniach, 1960). A similar relationship exists between the red cell precursors in the bone marrow and the peripheral megaloblasts or macrocytes in pernicious anaemia. As in this disease, the cause might be arrested maturation of the crypt cells and an actual slowing in certain phases of mitosis.

SUMMARY

Biopsies of the mucosa of the small intestine of 22 patients were subjected to light and electron microscopic studies. The patients were grouped into 'controls', coeliac disease, and idiopathic steatorrhoea, and in the last two groups the clinical condition and therapeutic progress was recorded. Electron microscopic studies were directed mainly towards the microvilli of the surface epithelium. It was found that the latter were decreased in height in coeliac disease and idiopathic steatorrhoea and showed greater irregularity in shape and spacing. A good correlation existed between the light microscope appearances of villous atrophy and decrease in surface cell height and the electron microscope findings of microvillous abnormalities.

Our thanks are due to the many physicians who kindly referred their patients to us for investigation, to Dr. R. A. B. Drury and Mr. J. E. Mayhew for the histology, and Mrs. S. Roberts for preparing the electron microscopy sections.

One of us (M.S.) is working with a grant from the Medical Research Council to which grateful acknowledgements are herewith made.

REFERENCES

Anderson, C. M. (1960). Histological changes in the duodenal mucosa in coeliac disease. *Arch. Dis. Childh.*, **35**, 419.

Brettauer, J., and Steinach, S. (1857). Untersuchungen über das Cylinderepithelium der Darmzotten und seine Beziehung zur fettresorption. S.-B. *Akad. Wiss. Wien, math.-nat. Kl.*, **23**, 303.

Doniach, I., and Shiner, M. (1957). Duodenal and jejunal biopsies II. Histology. *Gastroenterology*, **33**, 71.

Glauert, A. M., Rogers, G. E., and Glauert, R. H. (1956). A new embedding medium for electron microscopy. *Nature (Lond.)*, **178**, 803.

Granger, B., and Baker, R. F. (1949). Electron microscope investigation of the striated border of intestinal epithelium. *Anat. Rec.*, **103**, 459.

Hartman, R. S., Butterworth, C. E., Hartman, R. E., Crosby, W. H., and Shirai, A. (1960). An electron microscope investigation of the jejunal epithelium in sprue. *Gastroenterology*, **38**, 506.

Palade, G. E. (1952). A study of fixation for electron microscopy. *J. exp. Med.*, **95**, 285.

Palay, S. L., and Karlin, L. J. (1959). An electron microscopic study of the intestinal villus. I. The Fasting animal. *J. biophys. biochem. Cytol.*, **5**, 363.

Rubin, C. E., Brandborg, L. L., Phelps, P., Taylor, H. C. Jr., Murray, C.V., Stemler, R., Howrie, C., and Volwiler, W. (1960). Studies in coeliac disease II. *Gastroenterology*, **38**, 517.

Shiner, M. (1956). Duodenal biopsy. *Lancet*, **1**, 17 and 85.

—— (1960a). Coeliac disease. *Gut*, **1**, 48.

—— (1960b). *Histopathology in Coeliac Disease*. Proceedings of the International Congress of Gastroenterology, Leyden, Holland, April 20-24. In the press.

——, and Doniach, I. (1960). Histopathological studies in steatorrhea. *Gastroenterology*, **38**, 419.

Zetterquist, H. (1956). *The Ultrastructural Organisation of the Columnar Absorbing Cells of the Mouse Jejunum*. Thesis from the Department of Anatomy, Karolinska Institute, Stockholm, Sweden.

——, and Hendrix, T. R. (1960). *Bull. Johns Hopk. Hosp.*, **106**, 240.

CORRECTION

In Table I of the paper entitled 'A clinical appraisal of the treatment of chronic duodenal ulcer by vagotomy and gastric drainage operation' by W. Gerald Austen and Harold C. Edwards on page 158 of the June issues, the word 'pyloroplasty' has been incorrectly repeated. The relevant line of Table I should read:—

Type of operation performed with vagotomy	
Gastroenterostomy	90
Pyloroplasty	10

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Long-term medical management and complications of 'resistant' ascites

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SYNOPSIS This paper reports the experience of treating patients with hepatic cirrhosis and ascites with an aldosterone inhibitor in addition to conventional therapy. Good results are demonstrated in 13 patients previously resistant to treatment.

Ratnoff and Patek (1942) commented that ascites was the most frequent and characteristic sign of hepatic cirrhosis, and the serious prognosis of this complication was evident from the six-month survival of only 50% of their patients. Despite progress in therapy, the outlook remains extremely grave when the onset of ascites is insidious and when resistance develops to treatment with sodium restriction and standard diuretic agents (Sherlock, 1958). Most authorities agree with Blakemore (1952) that intractable ascites represents an end stage of cirrhosis and that such patients have at best but a few months to live. Available measures include paracentesis abdominis, a temporary expedient which may lead to protein and electrolyte depletion, hypotension, and coma (Sherlock, 1958), or various portal-systemic shunt operations (Blakemore, 1952; Linton, 1956; McDermott, 1960). The last reduce portal pressure and obstruction to hepatic venous or lymphatic flow but affect only mechanical aspects of the disease. Operative mortality with resistant ascites may exceed 40% (Linton, 1956), while the subsequent development of progressive hepatic failure or neuropsychiatric changes are also important considerations. Refractory ascites, therefore, is considered a strong contraindication to the surgical treatment of portal hypertension (Sherlock, 1958; Blakemore, 1952; Mackby, 1960; Walker, 1957), although newer techniques may justify some reappraisal (McDermott, 1960).

Since patients with cirrhosis and ascites have features of excess aldosterone activity (Luetscher and Johnson, 1954), the management of refractory fluid retention has been directed toward methods of combating hyperaldosteronism. Bilateral adrenalectomy (Marson, 1954; Giuseffi, Werk, Larson, Shiff, and Elliott, 1957) has been effective only occasionally, presumably because of the dangers of the procedure in patients with liver disease and the difficulties involved in immediate and long-term postoperative management. Medical suppression of adrenocortical function has been attempted (Summerskill and Crabbé, 1957; Stormont, Crabbé, Fast, Wolfe, and Davidson, 1959) but the toxicity of the drugs available precludes their long-term use. More recently, steroidal lactones (Kagawa, Cella, and van Arman, 1957), which act as chemical antagonists of aldosterone (Liddle, 1958), have been given by mouth without side-effects to patients with cirrhosis and have procured an initial diuresis, even when the ascites was refractory to standard measures (Clowdus, Higgins, Rosevear, and Summerskill, 1960; Henley, Streeten, and Pollard, 1960).

The progress of patients with resistant ascites treated medically is rarely specified after they leave the hospital and depends on many factors associated with hepatic disease, but particularly on their ability to adhere to a strict regimen of diet and drugs. In this paper, details of an effective long-term medical regimen to control ascites previously resistant to medical management by using an aldosterone antagonist in combination with standard diuretics are described, and the progress of 13 patients thus treated for periods up to two years is reported. Special attention is given to relaxation of the dietary restriction of sodium, which is made possible with treatment, to the influence of liver function in determining prognosis, and to the nature and management of complications arising during treatment.

[1]On assignment to the Mayo Foundation. The contents of this article are the personal views of the authors and are not to be construed as official Air Force policy nor as Air Force endorsement of any commercial product described

MATERIALS AND METHODS

Thirteen patients with cirrhosis (Table I) were treated at first in a hospital and were examined subsequently at intervals of two weeks to three months throughout the period of follow-up. The diagnosis of cirrhosis was made on clinical grounds, being supported by biochemical tests of liver function (Table I) and, in eight instances, by histological examination of hepatic tissue. Six patients (Cases 4, 5, 8, 9, 11, and 13) had been chronic alcoholics, but continued alcoholism had been a factor during the preceding two months in only one (Case 13). In the other patients, the cause of the cirrhosis and its possible relationship to previous hepatitis was uncertain (cryptogenic). Tense ascites had been present for periods of one month to three years despite treatment and was shown to be refractory for 10 days or longer to treatment with dietary restriction of sodium (less than 10 mEq. in 24 hours), and combinations of meralluride (mercuhydrin), chlorothiazide, or prednisone in the hospital. Nine patients had required paracenteses on two to 12 occasions during the preceding year, and

TABLE I

PATIENTS STUDIED AND RESULTS OF TREATMENT

Case	Age (yr.) and Sex		Duration of Ascites (mth.)	Previous History[1]			Loss of Weight in Hospital with Initial Treatment		Chemical Tests of Liver Function (a) Initial and (b) Latest Follow-up				Comments
				Coma	Haemorrhage[2]	Paracenteses	Amount (lb.)	Days	Serum Bilirubin (mg./100 ml.)	Serum Albumin (g./100 ml.)	Bromsulphalein Retention at 60 Minutes (%)	Prothrombin Time (sec.)	
1[2]	55	F	32	1	0	3	35	70	(a) 1·9	3·0	>30	23	Transient impending coma with chlorothiazide, otherwise well for 24 mth., taking diuretics daily and 80-mEq. sodium diet (see Fig. 2)
									(b) 1·3	3·4	>30	24	
2[2]	67	F	18	0	0	3	8	22	(a) 1·1	2·4	>30	22	Died after 2 mth. Azotaemia and terminal gastrointestinal haemorrhage
									(b) 1·0	3·8	>30	23	
3[2]	51	M	9	0	0	2	23	18	(a) 2·6	3·0	>30	22	Well for 22 mth., taking intermittent diuretic therapy and 10-mEq. sodium diet
									(b) 3·3	3·2	>40	23	
4	46	M	6	1	0	0	12	22	(a) 1·4	4·2	>30	22	Well for 15 mth. Transient hyponatraemia and azotaemia, slow improvement in hepatic function, no therapy necessary
									(b) 0·7	4·7	16	18	
5[2]	59	M	36	0	3	24	14	22	(a) 1·2	3·4	20	21	Well for 20 mth., taking intermittent diuretic therapy and 10-mEq. sodium diet
									(b) 0·7	4·8	20	19	
6[2]	69	M	3	0	0	0	65	32	(a) 1·4	3·8	30	24	Died after 6 mth. Hepatoma invading portal vein with re-accumulation of ascites and terminal gastrointestinal haemorrhage
									(b) 1·2	4·0	30	—	
7	61	M	2	1	0	1	32	30	(a) 2·3	2·4	30	22	Died of unrelated cause after 2 mth.
									(b) 2·1	2·6	30	19	
8[2]	61	M	1	0	—	—	33	20	(a) 4·1	2·7	—	21	Died after 1 mth. from gastrointestinal haemorrhage. Hepatoma found at necropsy
									(b) 5·9	2·8	—	—	
9[2]	64	F	12	1	0	5	16	11	(a) 1·2	3·7	>30	20	Well for 9 mth., taking diuretics and 40-80-mEq. sodium diet. Died in hepatic failure with azotaemia
									(b) 0·9	3·7	>30	—	
10[2]	44	M	24	1	0	4	22	19	(a) 0·9	3·2	>30	20	Died after 4 mth.: azotaemia and hyperkalaemia (Fig. 4)
									(b) 1·1	3·4	>30	21	
11	40	F	24	0	0	1	17	24	(a) 2·5	2·8	>30	22	Well for 15 mth. Slow improvement in liver function. No therapy now necessary
									(b) 0·6	5·0	16	18	
12[2]	62	F	7	1	0	6	23	24	(a) 3·6	2·7	>30	20	Died after 3 mth.: azotaemia and terminal gastrointestinal haemorrhage
									(b) 5·3	2·4	—	21	
13[2]	49	M	1	0	0	0	51	32	(a) 9·2	2·8	—	27	Well for 9 mth., taking 20-mEq. sodium diet. Recovered from gastrointestinal haemorrhage and azotaemia
									(b) 1·50	3·9	22	22	
							Normal values		<1·0	>3·5	< 5	17	

[1]All had been unresponsive to diet and diuretics previously
[2]Patients with gastro-oesophageal varices

10 had splenomegaly with oesophageal varices as evidence of portal hypertension. Post-mortem examinations were made on all patients who died.

During initial hospital treatment patients were given diets containing on analysis less than 10 mEq. of sodium and between 80 and 100 mEq. of potassium daily. Fluid intake was restricted only if azotaemia occurred. Weight was determined daily, and the volume, together with the sodium and potassium contents, of 278 specimens of 24-hour urine was determined during the treatment of patients 1 to 9. During the time in hospital and at follow-up appointments, chemical tests of liver function and concentrations of blood urea, together with serum sodium, potassium chloride, and bicarbonate, were measured at intervals by standard procedures. Determinations of ammonia in arterial blood using the method of Seligson and Hirahara (1957), and electroencephalographic recordings, which were graded 0 to 4 (Summerskill, Clowdus, and Casey, 1960a), were made on patients with neuropsychiatric changes. Exchangeable sodium, exchangeable potassium, and total body water were measured by using Na^{24}, K^{42}, and D_2O as tracer substances, as described elsewhere (Summerskill *et al.*, 1960a; Clowdus, II, Summerskill, Casey, Higgins, and Orvis, 1961).

PRELIMINARY ASSESSMENT AND RESPONSE TO INITIAL TREATMENT

Initially, all patients had evidence of severe hepatic disease with ascites and muscle wasting (Table I). Three patients were clinically jaundiced, one being deeply icteric. All had direct-reacting bilirubin in the serum. The concentration of serum albumin initially was 3 g. per 100 ml. or less in eight patients, and all who were not jaundiced had grossly abnormal retention of sulphobromophthalein (bromsulphalein). Serum glutamic oxalacetic transaminase activity and concentrations of globulin were abnormal in every instance. All patients were hypoprothrombinaemic, and the prothrombin time was unresponsive to treatment with vitamin K. Five patients (Cases 4, 7, 9, 10, and 13) had initial clinical and electroencephalographic evidence of impending hepatic coma, and two (Cases 1 and 10) had previously had coma following paracentesis. These considerations were felt to preclude any patient from consideration for surgical treatment.

After resistance of the ascites to a low-sodium diet with standard diuretic agents (mercuhydrin, chlorothiazide, and prednisone) had been confirmed, each patient was given medication with spirolactone-aldactone, 400 to 1,200 mg. in two to four doses daily in combination with chlorothiazide, 500 to 1,000 mg. in two doses daily; meralluride (mercuhydrin), 2 ml. daily intramuscularly; or prednisone, 30 mg. daily in three doses (Fig. 1). Adjustments of the dose and frequency of administration of each drug and of the most satisfactory combinations of

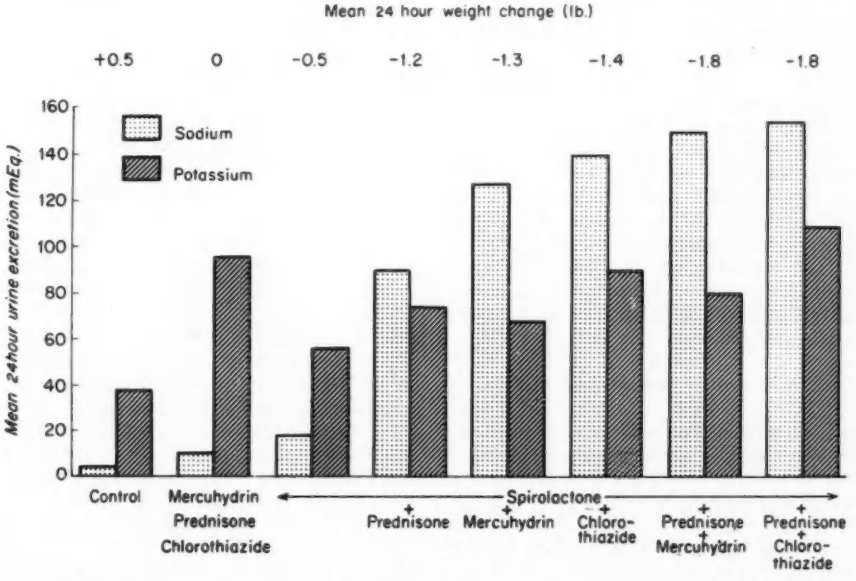

FIG. 1. *Mean change in weight, volume of urine, and urinary excretion of sodium and potassium in relation to treatment.*

drugs to produce a sustained diuresis were necessary in each patient.

All responded strikingly to treatment (Table I and Fig. 1), between 8 and 65 lb. in weight being lost in 12 to 42 days. The greatest loss of weight by an individual in 24 hours was 5½ lb., this being associated with a 24-hour excretion of 337 mEq. or sodium. There was usually a delay of a week, and sometimes considerably longer, before the diuresis began. With aldactone alone, a moderately increased amount of sodium was excreted in association with trivial loss of weight. The addition of prednisone or aldactone doubled loss of weight and increased excretion of sodium more than fourfold, while the use of aldactone in combination with chlorothiazide or mercuhydrin promoted comparable loss in weight but greater excretion of sodium. Two drugs were more effective than one in combination with aldactone; greater loss of weight and excretion of sodium occurred when either mercuhydrin or chlorothiazide was added to aldactone and prednisone. The greatest loss of weight (water) in relation to urinary excretion of sodium and potassium occurred when prednisone was administered with aldactone; the addition of thiomerin or chlorothiazdie to this combination of drugs caused a proportionately greater increase in excretion of the cations than loss of weight. Loss of potassium was greatest during regimens in which chlorothiazide was incorporated, and least when aldactone was administered. When aldactone and chlorothiazide were given together, a negative potassium balance often occurred.

Apart from improvement in jaundice in patient 13, there was no significant change in hepatic function tests during the period of initial diuresis. However, four of five patients who initially were in impending hepatic coma recovered during treatment, which included chlorothiazide, without antibiotic therapy or restriction of protein. No neuropsychiatric changes developed in the other patients. Complications arising during initial treatment, including those involving water and electrolyte metabolism, are discussed later.

RESULTS OF LONG-TERM MANAGEMENT

Long-term programmes of management after the patients left the hospital were adjusted to subsequent progress determined by their ability to continue a diet severely restricted in sodium and to abstain from alcohol, as well as by alterations in hepatic function and other complications incident to liver disease. Six patients (Cases 1, 3, 4, 5, 11, and 13) are alive and have remained free from significant fluid accumulation during periods of eight months to two years (mean 16 months). One patient (Case 7) died at home of an unrelated cause without re-accumulating ascites. Six patients died as a result of complications due to progressive liver disease and portal hypertension (Table I). The mean survival period in these cases was only four months, but gross ascites re-accumulated in only one (Case 6), this being due to occlusion of the portal vein by hepatoma.

Two patterns of response were defined during follow-up studies: (1) that of the patients who ultimately dispensed with diet and drugs, and (2) that of the patients who required continuous therapy. The second group of patients was subdivided into those who were able to continue strict sodium restriction at home (as shown by analysis of aliquots of their diets) and those in whom some relaxation of sodium intake was inevitable because of their inability to prepare or adhere to a correct diet. A trivial amount of ascites was allowed to accumulate in patients taking diuretics continuously as a precaution against sodium depletion.

PATIENTS RETURNING TO NORMAL DIETS WITHOUT DIURETIC THERAPY Three patients (Cases 4, 11, and 13) were chronic alcoholics in whom ascites had developed within the year preceding treatment but who ceased drinking one to three months before entering the hospital. After initial treatment, liver function improved in patient 13 only. All avoided further consumption of alcohol, and a diet providing approximately 10 mEq. of sodium, a liberal intake of protein (through low-sodium supplements), and added vitamins was prescribed. Subsequently, none reaccumulated fluid, all gained flesh, and hepatomegaly decreased. Chemical tests of liver function improved commensurately (Table I). The daily sodium content of the diet was increased cautiously by 20 mEq. increments at three-monthly intervals, and all have now reverted to normal diets without adverse effects, nine to 18 months having elapsed without retention of fluid.

PATIENTS REQUIRING CONTINUOUS TREATMENT The patients in this group can be subdivided into two groups according to whether they required intermittent or continuous diuretic therapy.

1 *Dietary Sodium of 10 mEq. Daily with Intermittent Diuretic Therapy* Two patients (Cases 3 and 5) were free from ascites when they left the hospital, and continued a diet providing approximately 10 mEq. of sodium without undue difficulty. Subsequently, fluid re-accumulated, with gains of 4 and 6 lb. within three months, and in addition to diet diuretics were necessary. After several adjustments, one patient (Case 3) has remained

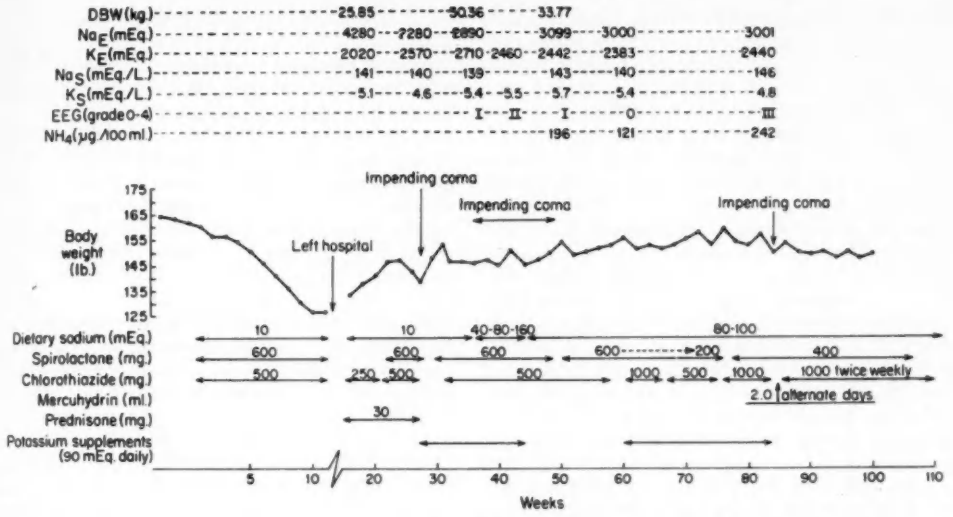

FIG. 2. *Details of treatment and complications in the long-term management of resistant ascites (patient 1). Diet and medication are related to body weight, dry body weight (DBW), exchangeable sodium (Na_E) and potassium (K_E) values; serium sodium (Na_S) and potassium (K_S) concentrations, and arterial ammonia concentrations (NH_4).*

free from ascites for 22 months with treatment for one week every two months with spirolactone, 600 mg. daily, to which prednisone, 30 mg. daily, and chlorothiazide, 500 mg. twice daily, has been added during the last three days of the week. Patient 5 has again become responsive to chlorothiazide and has maintained a steady weight for 19 months by taking 500 mg. of this drug on alternate days. On similar regimens, patient 7 remained free from significant ascites until his death, but patient 6 re-accumulated fluid at home terminally.

2 *Liberal Dietary Sodium with Continuous Diuretic Therapy* Five patients (Cases 1, 8, 9, 10, and 12) were unable technically or by inclination to persevere with strict dietary restriction of sodium. Consequently, fluid began to re-accumulate after discharge from the hospital. Studies of sodium balance (Clowdus *et al.*, 1960) showed that the daily administration of spirolactone in doses of 300 to 800 mg., together with other diuretics, permitted relaxation of the dietary restriction of sodium without undue retention or loss of sodium. Careful supervision with frequent readjustments of treatment was necessary, but with appropriate regimens all remained free from obvious ascites, despite diets containing 50 to 100 mEq. of sodium. Management sometimes involved difficulties, most of which are illustrated by the progress of patient 1 (Fig. 2).

After a good response to treatment in hospital and stabilization of weight on diet alone, this patient was unable to persevere with the diet at home and gained 7 lb. within six weeks. Chlorothiazide, prednisone and then spirolactone were prescribed in varying doses. She then lost weight, but an episode of impending coma, presumed to be due to chlorothiazide, necessitated withdrawal of drugs. Weight again increased; a return to treatment with aldactone and chlorothiazide was made with satisfactory diuresis, potassium supplements having been added to the regimen. However, an intermittent mild neuropsychiatric disorder persisted with minor abnormalities in the electroencephalogram.

An attempt was then made to provide more sodium in the diet. Balance studies with an 80-mEq. sodium diet showed a satisfactory sodium balance and no gain in weight, while treatment with diuretics was continued, but fluid accumulated with greater amounts of sodium. Accordingly, an 80-mEq. sodium diet with continued daily treatment with spirolactone and chlorothiazide was instituted satisfactorily. Attempts to stop chlorothiazide and later to reduce the dosage of aldactone slowly by 100 mg. were followed by increases in weight. Diuresis was procured by increasing the dose of chlorothiazide, but another transient episode of impending coma occurred. The dose of spirolactone was increased, but replacement of chlorothiazide by

injections of thiomerin on alternate days failed to retard the gain in weight. With a schedule of spirolactone, 400 mg. daily, with chlorothiazide given only twice weekly in doses of 500 mg. twice a day, the subsequent course has been satisfactory. Thus a palatable diet requiring little special preparation has been combined satisfactorily with continuous diuretic therapy which is less likely to cause complications.

Certain observations, which apply also to other patients, were evident from this study. A considerable proportion of the weight gained after leaving the hospital was due to increase in flesh, as shown by serial calculations of the dry body weight (DBW), which represents body weight less body water. Neuropsychiatric complications occurred specifically in relation to chlorothiazide therapy, but could not be related to potassium depletion, as values for exchangeable body potassium (K_E), K_E/DBW (78·1, 89·2 and 72·3 mEq./kg.), and concentrations of serum potassium (K_S) were not strikingly changed in the presence of clinical or electroencephalographic evidence of impending coma. Moreover, potassium supplements failed to protect against the onset of coma on two occasions and were not followed by striking increases in body potassium stores as shown by K_E measurements. In contrast, concentrations of ammonia in arterial blood were above the normal range (less than 100 mcg. per 100 ml.) and became more abnormal in association with the clinical and electroencephalographic changes of impending coma.

COMPLICATIONS

GASTROINTESTINAL HAEMORRHAGE AND HEPATOMA

Massive haemorrhage from gastro-oesophageal varices occurred in five patients, all of whom had severely impaired liver function with defective blood coagulation; only one survived (Table I). Bleeding occurred during initial hospital treatment in three patients (Cases 2, 8, and 13), in two of whom hepatoma was found at necropsy, despite previous failure to identify malignant cells in the ascitic fluid.

OVERHYDRATION, HYPERKALAEMIA, AND AZOTAEMIA

Disorders of water and electrolyte metabolism, sometimes with azotaemia, were prominent, either initially or during subsequent management (Tables I and II). Before treatment, isotope studies on 11 patients confirmed the excess total body water (TBW) and exchangeable sodium (Na_E) known to occur in patients with ascites (Birkenfeld, Liebman, O'Meara, and Edelman, 1958). Paradoxical hyponatraemia (Talso, Spafford, Ferenzi, and Jackson, 1956) was present at this stage in five patients who had serum concentrations of less than 135 mEq. of sodium per litre. Values for serum potassium (K_S), exchangeable body potassium (K_E), and blood urea were within the normal range, except in one patient who was hyperkalaemic and mildly azotaemic at this stage. Hyponatraemia was associated with hypochloraemia and reductions in serum bicarbonate concentrations. The lower initial values

TABLE II

CHANGES IN VALUES FOR DRY BODY WEIGHT (DBW), TOTAL BODY WATER (TBW), BODY EXCHANGEABLE SODIUM (Na_E) AND POTASSIUM (K_E), SERUM SODIUM (Na_S) AND POTASSIUM (K_S), AND BLOOD UREA (U_B) BEFORE AND AFTER DIURESIS

	DBW (kg.)		*TBW (kg.)*		*Na_E (mEq.)*		*Na_S (mEq./l.)*		*K_E (mEq.)*		*K_S (mEq./l.)*		*U_B (mg./100ml.)*	
Case	*Before*	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*
No azotaemia														
1	28·1	28·5	47·0	36·7	5,000	2,700	143	137	2,650	2,505	5·4	5·5	49	40
3	32·8	33·0	49·0	40·0	3,645	2,464	132	127	2,940	2,800	4·2	4·6	28	34
5	25·3	25·9	49·8	41·2	4,720	3,610	132	127	—	—	5·9	5·6	41	34
6	24·4	25·7	76·1	56·6	5,270	3,784	140	134	2,910	2,680	5·4	5·2	47	44
8	34·3	33·9	49·4	40·6	4,880	3,760	132	132	2,470	2,082	5·3	5·6	36	36
Mean	29·0	29·4	54·3	43·0	4,703	3,263	136	131	2,744	2,517	5·2	5·3	40	38
Percentage change	+ 1·4		− 20·7		− 30·6		− 3·7		− 8·2		+ 1·9		− 5·0	
Azotaemia														
2	36·3	35·1	39·4	37·6	3,930	2,290	137	108	2,050	2,100	5·3	6·8	59	153
4	17·8	18·0	48·2	46·2	4,550	3,263	135	119	2,050	2,080	4·5	4·7	37	90
9	20·2	19·6	32·9	30·3	2,751	2,330	140	136	1,851	1,949	5·6	6·5	78	132
10	24·7	25·2	48·5	39·3	3,617	2,702	134	115	2,750	2,600	6·5	8·2	54	110
12	23·1	23·4	41·7	32·8	3,616	2,407	130	117	1,574	1,263	4·0	6·2	33	123
13	26·8	25·8	50·9	35·4	5,756	2,338	138	102	2,384	2,169	3·3	5·7	12	111
Mean	24·8	24·5	43·6	36·9	4,037	2,555	136	116	2,110	2,027	4·9	6·4	45	120
Percentage change	− 1·2		− 15·4		− 36·7		− 14·7		− 3·9		+ 30		+ 166	

for total body water, Na_E, and K_E in patients subsequently having azotaemia reflected their smaller physiques as shown by the dry body weight. Otherwise the two groups could not be distinguished before treatment.

During diuresis, greater hyponatraemia developed, while three patients became azotaemic at this stage, and three more became azotaemic during subsequent management (Table II). The presence of azotaemia was associated with symptoms, more striking metabolic changes, and a worse prognosis. The clinical syndrome consisted of apathy, nausea, and anorexia, progressing in more severe instances to personality changes and stupor. The motor system disorder of impending coma (flapping tremor, hyporeflexia, rigidity, and clonus) was not prominent. Mental changes were not specific, and abnormalities of the electroencephalogram, arterial *p*H, and arterial ammonia concentrations, if present, were not striking and did not become more severe as the condition deteriorated (Summerskill *et al.*, 1960a). Azotaemia developed in the absence of precipitating factors, such as gastrointestinal haemorrhage or paracentesis abdominis, and without evidence of pre-existing renal disease, alterations in systemic blood pressure, and volume of urine (which were recorded daily), or characteristic histological changes in the kidneys at necropsy. A smaller proportion of body water was lost by azotaemic patients than by the other patients. Losses of sodium were comparable, while relatively little potassium was lost by either group. There was much greater hyponatraemia in azotaemic patients in association with the greater water retention, but concentrations of serum potassium became raised without elevation of body potassium content (K_E). These disorders were not related to changes in flesh, as little alteration occurred in dry body weight in either group (Table II).

The patients who became azotaemic often had more severe liver disease on clinical assessment and more grossly deranged chemical tests of liver function (Table I). Two (Cases 4 and 13) recovered simultaneously with improvement in liver function, whereas azotaemia, hyponatraemia, and hyperkalaemia persisted for periods of two to nine months before death in three other patients (Cases 9, 10, and 12). Sudden death of three azotaemic patients (Cases 2, 9, and 10) appeared to be primarily due to hyperkalaemia, as electrocardiograms showed characteristic abnormalities and no alternative explanation could be found at necropsy. There was no relationship between the type or amount of drugs prescribed and the onset of azotaemia; patients subsequently having this complication had received smaller quantities of drugs and had a slower diuretic response.

Treatment of overhydration and azotaemia was unsatisfactory. Hyponatraemia without raised concentrations of serum potassium or blood urea gave rise to no specific symptoms and corrected itself spontaneously after diuresis. In azotaemic patients, overhydration was controlled temporarily by restriction of fluids, but severe thirst made this difficult for longer-term use, and azotaemia progressed notwithstanding (Cases 2, 9, 10, and 12). The enhanced water diuresis with prednisone (30 to 45 mg. daily) was not sufficient to prevent the onset or progression of overhydration and azotaemia in five patients, nor did the administration of mannitol (5% solution) by vein cause weight loss in five patients already receiving diuretics. An increased urinary volume followed mannitol infusions but was accounted for by the fluid load administered. Four patients excreted an increased amount of sodium in the subsequent 48 hours (mean 20·5 mEq. of sodium per litre of mannitol administered).

HEPATIC COMA In addition to the neuropsychiatric changes during azotaemia, hepatic coma occurred as a terminal event following gastrointestinal haemorrhage in Cases 2, 6, and 8. Personality changes, non-specific tumour, and minor electroencephalographic abnormalities of grade 1 or 2 severity appeared to be due primarily to the severity of the liver disease on initial assessment in Cases 4, 9, 10, and 13. In Cases 1 (Fig. 2) and 7 transient impending coma was clearly related to treatment with chlorothiazide and responded quickly to withdrawal of the drug. In other cases, diuretic therapy, including chlorothiazide, had no direct influence on the neuropsychiatric changes, and three patients recovered during good diuretic responses.

Several problems associated with management during the development and course of disorders of water and electrolyte metabolism and azotaemia are illustrated by the progress in Case 10 (Fig. 3). Prolonged treatment with sodium restriction and increasing doses of diuretics were necessary to initiate diuresis. After steady loss of weight, apathy, confusion, and anorexia appeared in association with more severe hyponatraemia and hyperkalaemia, neither of which could be explained from changes in body composition as there was no increase in hydration (as indicated by the ratios $Na_E + K_E$/TBW) and no evidence of sodium depletion or potassium retention from the Na_E and K_E values. The electrocardiogram showed gross changes of hyperkalaemia. Simultaneously, azotaemia developed, with deterioration in the electroencephalogram and abnormal arterial concentrations of

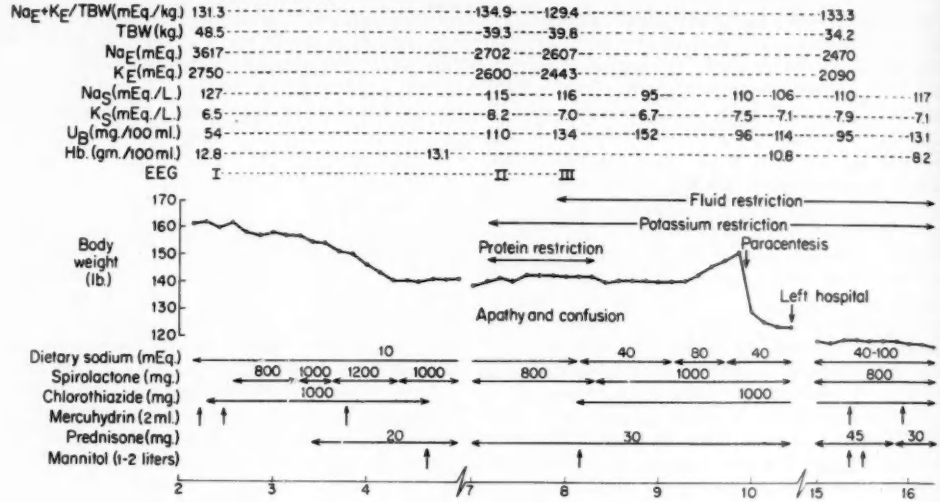
Na_E+K_E/TBW(mEq./kg.) 131.3 134.9 129.4 133.3
TBW(kg.) 48.5 39.3 39.8 34.2
Na_E(mEq.) 3617 2702 2607 2470
K_E(mEq.) 2750 2600 2443 2090
Na_S(mEq./L.) 127 115 116 95 110 106 110 117
K_S(mEq./L.) 6.5 8.2 7.0 6.7 7.5 7.1 7.9 7.1
U_B(mg./100 ml.) 54 110 134 152 96 114 95 131
Hb.(gm./100 ml.) 12.8 13.1 10.8 8.2
EEG I II III
Fluid restriction
Potassium restriction
Protein restriction
Paracentesis
Left hospital
Apathy and confusion
Body weight (lb.)
170
160
150
140
130
120
Dietary sodium (mEq.) 10 40 80 40 40-100
Spirolactone (mg.) 800 1000 1200 1000 800 1000 800
Chlorothiazide (mg.) 1000 1000
Mercuhydrin (2 ml.)
Prednisone (mg.) 20 30 45 30
Mannitol (1-2 liters)
2 3 4 7 8 9 10 15 16
Weeks

FIG. 3. *Disorders of water and electrolyte metabolism and azotaemia during treatment of resistant ascites (patient 10). Changes in total body water (TBW), exchangeable sodium (Na_E) and exchangeable potassium (K_E) values; serum sodium (Na_S) and potassium (K_S) concentrations; and haemoglobin (Hb) and blood urea (U_B) concentrations, in relation to management.*

ammonia, but without change in blood pressure or output of urine. The neuropsychiatric disorder was not influenced by withdrawal of protein and chlorothiazide, but restriction of potassium resulted in some improvement in serum potassium concentration and in the electrocardiographic pattern. Relative overhydration then occurred, with a fall in the ratio, $Na_E + K_E$/TBW. Despite restriction of fluid, the concentration of serum sodium continued to fall and azotaemia increased, although improvement in the $Na_E + K_E$/TBW ratio occurred subsequently.

With improvement in the general condition, an increased dietary intake of sodium was attempted in conjunction with higher doses of spirolactone and chlorothiazide, so as to relax dietary restrictions before discharge from the hospital. The weight remained stable when a 40-mEq. sodium diet was used, although the concentration of serum sodium continued to fall and that of the blood urea rose, whereas an 80-mEq. sodium diet resulted in accumulation of fluid. Improvement in serum sodium and blood urea concentrations occurred, and to facilitate discharge from the hospital, paracentesis was performed uneventfully.

The further course was satisfactory only in relation to the fact that no further weight was gained. Precautions against excess intakes of water and potassium remained necessary, and serum potassium concentrations remained dangerously high, while hyponatraemia persisted and concentrations of blood urea fluctuated but never reverted to normal. On four occasions, mannitol was given without effect. Progressive anaemia occurred in association with the azotaemia; tests for occult blood in the stools had been negative on 12 occasions. There was no alteration of serum bilirubin concentration or other evidence of increased haemolysis, and blood smears, together with leucocyte and platelet counts, were unremarkable. The anaemia appeared to be associated with the impaired renal function, and blood transfusions were necessary. Long-standing constipation was aggravated by fluid restriction, and evacuation of faeces under caudal anaesthesia was necessary. Three days later the patient complained suddenly of progressive paralysis and died within five minutes, the mode of death being ascribed to hyperkalaemia, as there was no other explanation for it at post-mortem examination.

COMMENT

The long-term medical management of ascites has been emphasized less than immediate hospital treatment, although successful therapy depends particularly on perseverance with an unpalatable

and technically difficult diet, while there is understandably little information available regarding the progress of patients when ascites is refractory to treatment. In our experience, the use of spirolactone in combination with other diuretics always overcame the initial resistance of ascites to other medical measures, although there was occasionally a delay of two weeks before diuresis began. Moreover, this treatment permitted the relaxation of dietary restriction of sodium (Clowdus *et al.*, 1960) when necessary in subsequent management without fluid re-accumulating. The initial diuretic response was related to the number of drugs and the doses prescribed, but long-term management was achieved with less medication, the amount usually varying with the intake of sodium. Balance studies confirmed earlier observations regarding the metabolic effects of these various drugs. Spirolactone had a potassium conserving effect, whereas chlorothiazide increased the loss of potassium, and prednisone increased the excretion of water in relation to sodium and potassium (Clowdus *et al.*, 1960; Cattan and Vesin, 1957; Dingman, Finkenstaedt, Laidlaw, Renold, Jenkins, Merrill, and Thorn, 1958; Shaldon, McLaren, and Sherlock, 1960; Laragh, Heinemann, and Demartini, 1958; Read, Laidlaw, Haslam, and Sherlock, 1959; Salassa, Mattox, and Power 1958). Nevertheless, the simultaneous administration of aldactone and chlorothiazide often led to a negative potassium balance, while the increased diuresis of water associated with prednisone therapy did not necessarily prevent overhydration. The incidence of neuropsychiatric complications attributable to chlorothiazide in these and other patients treated by us was much below that reported by Read *et al.* (1959), and potassium depletion was not proved to be the major factor. Concentrations of serum potassium are infrequently reliable indices of body stores of potassium in patients with ascites (Summerskill *et al.*, 1960a; Clowdus, II, *et al.*, 1961; Talso *et al.*, 1956), and the direct effect of chlorothiazide on ammonia metabolism (Owen, Flanagan, and Tyor, 1959; Mackie, Stormont, Hollister, and Davidson, 1958; Casey, Summerskill, and Orvis, 1961) seen in one of the patients reported herein was supported by the demonstration of an interrelationship between ammonia and potassium metabolism in others (Casey *et al.*, 1961). This may explain the susceptibility of patients who had previously experienced coma to neuropsychiatric changes during chlorothiazide therapy and the raised arterial concentrations of ammonia under such circumstances, which is evident from earlier data (Read *et al.*, 1959). These considerations were of greatest importance during periods of rapid diuresis and when complications involving water and electrolyte metabolism occurred, but they seldom influenced long-term management because of the smaller doses of drugs necessary for maintenance. A combination of spirolactone and chlorothiazide was most convenient for long-term treatment but newer drugs may have fewer disadvantages.

The constant response to a drug acting primarily as an antagonist of aldosterone indicates the importance of hyperaldosteronism in the refractoriness of ascites to standard treatment. Such resistance appeared to be closely related to the severity of the liver disease, as patients in whom liver function ultimately improved dispensed with treatment, whereas others required continued diet and therapy with diuretics. A direct relationship between hyperaldosteronism and liver function (Summerskill and Crabbé, 1957; Coppage, Island, Cooner, and Liddle, 1961) or other factors important in the formation of ascites, however, remains speculative.

Resistant ascites associated with cirrhosis carried a more favourable long-term prognosis in the alcoholic than in the non-alcoholic, as greater recovery of liver function was possible as a result of a nutritious diet and abstinence from alcohol (Summerskill, Davidson, Dible, Mallory, Sherlock, Turner, and Wolfe, 1960b). Nevertheless, relaxation of diuretic therapy and sodium restriction had to be gradual in the alcoholic. In contrast, patients with cirrhosis of uncertain cause pursued a more inexorable course (Summerskill *et al.*, 1960b) which was reflected by their need for continued treatment to prevent re-accumulation of ascites. Only one patient became responsive after treatment to a diuretic which had been ineffective previously.

The prognosis is grave in resistant ascites because of several complications associated with advanced liver disease. Haemorrhage from ruptured gastro-oesophageal varices, hepatoma, and hepatic coma occurred during the course of follow-up. Disorders of water and electrolyte metabolism of uncertain origin occurred particularly frequently and constituted perhaps the greatest problem in management. Their high incidence may support an often assumed relationship to the use of a sodium-restricted diet and diuretics. No direct connexion between the various components of treatment and these complications could be demonstrated, however, and we were particularly careful to guard against sodium or potassium depletion. Advances in the management of other complications associated with hepatic disease have resulted in the greater prominence of disorders of water and electrolyte metabolism in hepatic failure (Chalmers, 1960), and their relationship to deterioration in hepatic function appears to be most important, especially as two patients with severe hyponatraemia and

azotaemia recovered when the liver disease improved.

Two uncertainly related phases were distinguished in this disorder, the first reflecting overhydration (Clowdus, II, *et al.*, 1961; Birkenfeld *et al.*, 1958; Talso *et al.*, 1956) and the second comprising more severe overhydration in association with azotaemia and additional metabolic and clinical changes (Clowdus, II, *et al.*, 1961). Hyponatraemia represented primarily dilution due to a relatively greater excess of body water than sodium, rather than sodium depletion; hypokalaemia, when present, may reflect the same phenomenon as well as deficiency of potassium. However, potassium supplements infrequently lead to replenishment of potassium stores as judged by changes in body potassium (Casey *et al.*, 1961), nor were they always effective in preventing coma during treatment with chlorothiazide. In addition to sodium retention, the relative excess of body water may result from diminished free water clearance (Schedl and Bartter, 1960) or excess ADH activity (Ralli, Robson, Clarke, and Hoagland, 1945) and it was particularly prominent when azotaemia developed. Although prednisone (Dingman *et al.*, 1958) and mannitol (Shaldon *et al.*, 1960; Schedl and Bartter, 1960) may enhance free water clearance in some patients, there was no significant clinical effect in our azotaemic group, perhaps because sodium diuresis was already in progress. Restriction of fluids limited overhydration most effectively but failed to halt azotaemia and caused great thirst.

Despite overhydration and consequent dilution, hyperkalaemia occurred with azotaemia and appeared important as a cause of death. It could not be related to retention of potassium, thus raising the possibility that redistribution of potassium from the intracellular to the extracellular compartments resulted in hyperkalaemia and contributed to hyponatraemia. Potassium restriction was not fully effective, and the use of insulin, glucose, and sorbitol in the treatment of hyperkalaemia must be considered also.

The impairment of renal function leading to azotaemia in our cases occurred without clinical or histological evidence of renal disease. A disorder of the renal circulation has been inferred by Papper, Belsky, and Bleifer (1959) and Hecker and Sherlock (1956), although oliguria and hypotension were not invariably present in their patients. In our cases, azotaemia preceded detectable oliguria and peripheral vascular collapse, but renal plasma flow was low (Feichter, Summerskill, and Wakim; unpublished data), as in three cases reported by Papper *et al.* (1959). This appeared the most important cause of a diminished glomerular filtration rate, but the anatomical and pathological basis is conjectural. The occurrence of progressive anaemia in one azotaemic patient was of interest in view of the relationships between erythropoiesis and renal function. The poor immediate prognosis of severe hyponatraemia with azotaemia stressed by others (Papper *et al.*, 1959; Hecker and Sherlock, 1956) was not altogether supported in our group, as these changes sometimes persisted for weeks or months and two patients recovered.

Secondary impairment of renal function may modify the clinical picture. The neuropsychiatric changes in azotaemic patients were not characteristic of hepatic coma and did not respond strikingly to restriction of protein and antibiotics. Abnormalities of the electroencephalogram, arterial *p*H, and blood ammonia concentration may be influenced by disorders of water and electrolyte metabolism and azotaemia (Chalmers, 1960; Webster and Gabuzda, 1959), thus making separation of clinical patterns difficult in patients with severe liver disease.

Our patients had hepatic disease of a severity which precluded initial consideration of surgical treatment and militated against the successful medical or surgical management of any subsequent gastrointestinal haemorrhage. The therapeutic approach of choice to resistant ascites, therefore, must be evaluated in relation to improvements in conservative management, as well as in surgical techniques and results (McDermott, 1960; Welch, Welch, and Carter, 1959; Barker and Reemtsma, 1960). The simultaneous improvement in liver function and portal hypertension (Reynolds, Geller, Kuzma, and Redeker, 1960) in cirrhosis of the alcoholic suggests that operation may be deferred for six months, so that response to medical treatment and the sincerity of abstention from alcohol can be assessed. Post-operative improvement in ascites may on occasion be related to abstinence from alcohol rather than to the operation itself, whereas hazardous operations are seldom warranted when continued alcoholism leads to hepatic failure. Until the various factors responsible for resistance of ascites in different patients are more clearly defined, surgical treatment would appear to be most clearly indicated for the relatively uncommon patient in whom resistant ascites is associated with apparently good liver function, the latter being judged particularly by general nutrition, the absence of jaundice or previous coma, and satisfactory blood coagulation. The demonstration of gastro-oesophageal varices would favour a portal-systemic shunt, particularly if gastrointestinal haemorrhage had occurred previously, whereas adrenalectomy might be considered for the other patients. In regard to paracentesis, Copland (1844-58) commented a century ago that it 'seems calculated to increase the mischief, and to

diminish the chances of a complete cure. It is the last means to which recourse should be had'.

SUMMARY

Resistance of ascites associated with hepatic cirrhosis to standard treatment was demonstrated in 13 patients, all of whom responded to treatment in a hospital with dietary restriction of sodium and a combination of diuretic agents, which included spirolactone. The effects of various combinations of drugs on excretion of water, sodium, and potassium were studied. Medical supervision and treatment were continued for periods of up to two years, and the following patterns of response in relation to the long-term management of resistant ascites were found.

Patients in whom alcoholism had been a factor in the previous six months were ultimately able to dispense with treatment, but only after several months. This was associated with continued abstention from alcohol and slow improvement in liver function. In patients with cirrhosis of uncertain cause, liver function either deteriorated or remained unchanged, and continuous or intermittent treatment was necessary. The amount of drugs required depended on patients' ability to adhere to a low-sodium diet. In most instances, it was necessary to relax the degree of sodium restriction. Continuous treatment with spirolactone and other drugs permitted a more liberal intake of sodium when necessary. The details and complications of management are described with particular emphasis on those involving water and electrolyte metabolism.

Six patients have remained well and free from disabling ascites, and six have died from complications associated with progressive liver disease; one patient died from an unrelated cause. Now that it is feasible to treat resistant ascites for long periods on an out-patient basis with a medical programme, the management of this condition, especially in regard to surgical procedures, is re-evaluated.

REFERENCES

Barker, H. G., and Reemtsma, K. (1960). The portacaval shunt operation in patients with cirrhosis and ascites. *Surgery*, **48**, 142-152.

Birkenfeld, L. W., Leibman, J., O'Meara, M. P., and Edelman, I. S. (1958). Total exchangeable sodium, total exchangeable potassium, and total body water in edematous patients with cirrhosis of the liver and congestive heart failure. *J. clin. Invest.*, **37**, 687-698.

Blakemore, A. H. (1952). Portacaval shunting for portal hypertension. *Surg. Gynec. Obstet.*, **94**, 443-454.

Casey, T. H., Summerskill, W. H. J., and Orvis, A. L. (1961). Disorders of potassium metabolism in liver disease. (Abstr.) *J. clin. Invest.*, **40**, 1028-1029.

Cattan, R., and Vesin, P. (1957). État actuel du traitement des cirrhoses ascitiques par la delta-cortisone. *Sem. Hôp. Paris*, **33**, 76-79.

Chalmers, T. C. (1960). Pathogenesis and treatment of hepatic failure. *New Engl. J. Med.*, **363**, 23-30; 77-82.

Clowdus, B. F., Higgins, J. A., Rosevear, J. W., and Summerskill, W. H. J. (1960). Treatment of 'refractory' ascites with a new aldosterone antagonist in patients with cirrhosis. *Proc. Mayo Clin.*, **35**, 97-105.

——, II, Summerskill, W. H. J., Casey, T. H., Higgins, J. A., and Orvis, A. L. (1961). Isotope studies of the development of water and electrolyte disorders and azotaemia during the treatment of ascites. *Gastroenterology*, **41**, 360-370.

Copland, James (1844-58). *A Dictionary of Practical Medicine*. Longman, Brown. Green, Longmans and Roberts, London.

Coppage, W. S., Island, D., Cooner, A. E., and Liddle, G. W. (1961). Metabolism of aldosterone in normal subjects and patients with cirrhosis (Abstr.) *Clin. Res.*, **9**, 177.

Dingman, J. F., Finkenstaedt, J. T., Laidlaw, J. C., Renold, A. E., Jenkins, D., Merrill, J. P., and Thorn, G. W. (1958). Influence of intravenously administered adrenal steroids on sodium and water excretion in normal and Addisonian subjects. *Metabolism*, **7**, 608-623.

Feichter, R. A., Summerskill, W. H. J., and Wakim, K. G. Unpublished data.

Giuseffi, J., Werk, E. E., Jr., Larson, P. U., Shiff, L., and Elliott, D. W. (1957). Effect of bilateral adrenalectomy in a patient with massive ascites and postnecrotic cirrhosis. *New Engl. J. Med.*, **257**, 796-803.

Hecker, R., and Sherlock, Sheila (1956). Electrolyte and circulatory changes in terminal liver failure. *Lancet*, **2**, 1121-1125.

Henley, K. S., Streeten, D. H. P., and Pollard, H. M. (1960). Hyperaldosteronism in liver disease: The treatment of refractory ascites by adrenalectomy and by the administration of spirolactones. *Gastroenterology*, **38**, 681-689.

Kagawa, C. M., Cella, J. A., and van Arman, C. G. (1957). Action of new steroids in blocking effects of aldosterone and deoxycorticosterone on salt. *Science*, **126**, 1015-1016.

Laragh, J. H., Heinemann, H. O., and Demartini, F. E. (1958). Effect of chlorothiazide on electrolyte transport in man: Its use in the treatment of edema of congestive heart failure, nephrosis, and cirrhosis. *J. Amer. med. Ass.*, **166**, 145-152.

Liddle, G. W. (1958). Aldosterone antagonists. *A.M.A. Arch. intern. Med.*, **102**, 998-1004.

Linton, R. R. (1956). Portal hypertension. In Schiff, Leon: *Diseases of the Liver*, pp. 195-233. Lippincott, Philadelphia.

Luetscher, J. A., Jr., and Johnson, B. B. (1954). Observations on the sodium-retaining corticoid (aldosterone) in the urine of children and adults in relation to sodium balance and edema. *J. clin. Invest.*, **33**, 1441-1446.

Mackby, M. J. (1960). Techniques of portal decompression. *Int. Abstr. Surg.*, **110**, 521-526.

Mackie, J. E., Stormont, J. M., Hollister, R. M., and Davidson, C. S. (1958). Production of impending hepatic coma by chlorothiazide and its prevention by antibiotics. *New Engl. J. Med.*, **259**, 1151-1156.

Marson, F. G. W. (1954). Total adrenalectomy in hepatic cirrhosis with ascites. *Lancet*, **2**, 847-848.

McDermott, W. V., Jr. (1960). The double portacaval shunt in the treatment of cirrhotic ascites. *Surg. Gynec. Obstet.*, **110**, 457-469.

Owen, E. E., Flanagan, J. F., and Tyor, M. P. (1959). Kidney as a source of blood ammonia: Effect of chlorothiazide. *Proc. Soc. exp. Biol. (N.Y.)*, **102**, 696-697.

Papper, S., Belsky, J. L., and Bleifer, K. H. (1959). Renal failure in Laennec's cirrhosis of the liver. I. Description of clinical and laboratory features. *Ann. intern. Med.*, **51**, 759-773.

Ralli, Elaine P., Robson, J. S., Clarke, Delphine, and Hoagland, C. L. (1945). Factors influencing ascites in patients with cirrhosis of the liver. *J. clin. Invest.*, **24**, 316-325.

Ratnoff, O. D., and Patek, A. J., Jr. (1942). The natural history of Laennec's cirrhosis of the liver. *Medicine (Baltimore)*, **21**, 207-268.

Read, A. E., Laidlaw, J., Haslam, R. M., and Sherlock, Sheila (1959). Neuropsychiatric complications following chlorothiazide therapy in patients with hepatic cirrhosis: Possible relation to hypokalaemia. *Clin. Sci.*, **18**, 409-423.

Reynolds, T. B., Geller, H. M., Kuzma, O. T., and Redeker, A. G. (1960). Spontaneous decrease in portal pressure with clinical improvement in cirrhosis. *New Engl. J. Med.*, **263**, 734-739.

Salassa, R. M., Mattox, V. R., and Power, M. H. (1958). Effect of an aldosterone antagonist on sodium and potassium excretion in primary hyperaldosteronism. (Letter to the Editor.) *J. clin. Endocr.*, **18**, 787-789.

Schedl, H. P., and Bartter, F. C. (1960). An explanation for and experimental correction of the abnormal water diuresis in cirrhosis. *J. clin. Invest.*, **39**, 248-261.

Seligson, David, and Hirahara, Katsugi. (1957). The measurement of ammonia in whole blood, erythrocytes, and plasma. *J. Lab. clin. Med.*, **49**, 962-974.

Shaldon, S., McLaren, J. R., and Sherlock, S. (1960). Resistant ascites treated by combined diuretic therapy (spironolactone, mannitol, and chlorothiazide). *Lancet*, **1**, 609-613.

Sherlock, Sheila (1958). *Diseases of the Liver and Biliary System*, 2nd ed. Blackwell, Oxford.

Stormont, J. M., Crabbé, J., Fast, B. Wolfe, S. J., and Davidson, C. S. (1959). The effect of prednisone and amphenone on fluid and electrolyte balance and on aldosterone excretion of patients with cirrhosis and ascites. *J. Lab. clin. Med.*, **53**, 396-416.

Summerskill, W. H. J., and Crabbé, J. (1957). Effect of amphenone therapy on urinary excretion of aldosterone and sodium in hepatic cirrhosis with ascites. *Lancet*, **2**, 1091-1095.

——, Clowdus, B. F., and Casey, T. H. (1960a). Clinical and metabolic changes during the development of azotemia in hepatic failure with ascites. *Proc. Mayo Clin.*, **35**, 783-791.

——, Davidson, C. S., Dible, J. H., Mallory, G. K., Sherlock, S., Turner, M. D., and Wolfe, S. J. (1960b). Cirrhosis of the liver: A study of alcoholic and non-alcoholic patients in Boston and London. *New Engl. J. Med.*, **262**, 1-9.

Talso, P. J., Spafford, Norma, Ferenzi, G., and Jackson, Helen O. (1956). Paradoxical hyponatremia associated with congestive heart failure and with cirrhosis of the liver. *Metabolism*, **5**, 58-69.

Walker, R. M. (1957). Portacaval anastomosis. *Lancet*, **1**, 57-59.

Webster, L. T., Jr., and Gabuzda, G. J. (1959). Relation of azotemia to blood "ammonium" in patients with hepatic cirrhosis. *A.M.A. Arch. intern. Med.*, **103**, 15-22.

Welch, C. S., Welch, H. F., and Carter, J. H. (1959). The treatment of ascites by side to side portacaval shunt. *Ann. Surg.*, **150**, 428-440.

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Serum haptoglobin in liver disease

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SYNOPSIS Serum haptoglobin levels have been measured in 115 cases of widely differing liver disease. Although low levels were found in some cases of cirrhosis and a number of patients with obstructive jaundice had increased levels, 70% of the values fell within the normal range. The estimation was of no help in distinguishing between intra- and extrahepatic obstructive jaundice. A characteristic pattern was observed in infective hepatitis, and a falling serum haptoglobin in the presence of increasing jaundice is diagnostic of the latter condition. The cause of these changes is uncertain. Low levels could not be accounted for by increased red cell breakdown and there was no correlation with the serum albumin level.

Prednisolone therapy produced a rise in serum haptoglobin level in cirrhosis, which was accompanied by an improvement in liver function. Temporary rises in level were observed in intrahepatic obstructive jaundice and were probably due to a non-specific effect of prednisolone. In the latter condition norethandrolone therapy was often associated with high levels.

Haptoglobin is an α_2 globulin which has the specific property of combining stoichiometrically with haemoglobin (Jayle, Boussier, and Tonnelat, 1956). The complex formed cannot pass through the glomerulus and the level of haptoglobin is an important factor in determining the renal threshold for haemoglobin. It is not surprising to find a reduced serum haptoglobin level in cases of intravascular haemolysis although the mechanism for the reduction in cases of haemolytic disease with extravascular erythrocyte destruction is not known (Brus and Lewis, 1959; Owen, de Gruchy, and Smith, 1960). The variations in level encountered in other diseases are also difficult to interpret, particularly since fever (Nyman, 1959) and non-specific stresses (Owen, Podanyi, and Smith, 1961a) may cause raised values. Low serum haptoglobin values have been reported in some cases of cirrhosis and raised levels in obstructive jaundice (Jayle and Vallin, 1952; Nyman, 1959; Owen, Mackay, and Got, 1959). Recently, Owen *et al.* (1961b) have stressed the value of this estimation in the differential diagnosis of jaundice; as a single test it proved slightly more effective than the serum alkaline phosphatase, albumin, or flocculation tests.

This paper describes findings in widely differing cases of liver disease. Serial estimations have been performed since little attention has previously been paid to spontaneous fluctuations, which could seriously affect the value of a single estimation. The changes in serum haptoglobin observed have been related to the degree of haemolysis present and the effect of prednisolone and other therapy has been assessed.

PATIENTS STUDIED

One hundred and fifteen cases were studied. These were divided into five main groups:—Cirrhosis (40), obstructive jaundice (33), infective hepatitis (27), congenital hyperbilirubinaemia (10), and miscellaneous (5), as shown in Table I. Two cases of portal cirrhosis and one alcoholic patient with cirrhosis are included in the group of intrahepatic obstruction since they were deeply jaundiced with all the features of cholestasis. Indeed the two patients with portal cirrhosis had initially been mistaken for cases of primary biliary cirrhosis.

METHODS

Haptoglobin was estimated by adding standard methaemoglobin solution to serum and determining the peroxidase activity of the resulting methaemoglobin complex colorimetrically (Owen, Better, and Hoban, 1960). Haptoglobin concentration is expressed in terms of bound methaemoglobin (M.Hb). The mean value for 31 normal subjects was found to be 81·0 mg./100 ml. with a range (mean $\pm$ 2 S.D.) of 8 to 154 mg./100 ml.

TABLE I

PATIENTS STUDIED

Diagnosis	*No. of Cases*
Cirrhosis	
Post-hepatitis or cryptogenic	19
Alcoholic	6
Juvenile	5
Haemochromatosis	10
Obstructive jaundice (intrahepatic)	
Primary biliary cirrhosis	18
Chronic chlorpromazine jaundice	1
Portal cirrhosis with cholestasis[1]	2
Alcoholic cirrhosis with cholestasis[1]	1
Unexplained	2
Obstructive jaundice (extrahepatic)	
Carcinoma	6
Stricture	3
Infective hepatitis	27
Congenital hyperbilirubinaemia	
Gilbert	8
Dubin-Johnson	2
Miscellaneous	
Secondary biliary cirrhosis	1
Cirrhosis with hepatocellular carcinoma	2
Congenital hepatic fibrosis	2
Total	115

[1]See text.

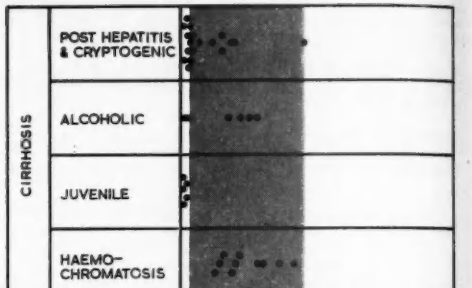

FIG. 1. *Serum haptoglobin levels in cirrhosis and obstructive jaundice*
× = *Norethandrolone therapy.*
The hatched area indicates the normal range.

This agrees reasonably well with the series of 152 normals studied by Smith and Owen (1961), which gave a mean value of 93 mg./100 ml. with a range (mean ± S.D.) of 13 to 173 mg./100 ml. In some cases the serum was also examined by starch gel electrophoresis using the vertical technique of Smithies (1955). The concentrations of the serum protein fractions were determined by the biuret method together with electrophoresis and scanning. Serum total bilirubin (Malloy and Evelyn, 1937) and serum alkaline phosphatase (King and Wootton, 1959) have also been measured.

Red cell survival and blood volume were determined by the radioactive chromium technique (Veall and Vetter, 1958). The results were expressed in terms of the Cr^{51} red cell half-life ($t_{\frac{1}{2}}$) and were not corrected for chromium elution. An estimate of peripheral haemoglobin destruction was obtained by multiplying the fraction of red cells destroyed daily by the total circulating haemoglobin. Since the chromium red cell half-life is less than the true half-life, the calculation overestimates the amount of haemoglobin destroyed but should give comparable values for different subjects. In a normal person the haemoglobin destruction rate is approximately 250 mg./kg./day.

RESULTS

1 CIRRHOSIS Serum haptoglobin was reduced (<8 mg./100 ml.) in six of the 19 patients with post-hepatitis or cryptogenic cirrhosis. In the others the level was usually in the lower part of the normal range, 11 cases having a value of less than 20 mg./100 ml. (Fig. 1). In general the patients with liver cell decompensation, as shown by ascites or jaundice, had a low serum haptoglobin and the well-compensated cirrhotic patients had a higher level. There were, however, many exceptions; for instance, three of the 11 patients with a haptoglobin level of less than 20 mg./100 ml. were out-patients with a well-compensated cirrhosis and two cases with levels of 66 and 71 mg./100 ml. had ascites and impending hepatic coma. This also applied to the alcoholic patients with cirrhosis. Two of the six cases studied had reduced levels; one of these was clinically well, the other in terminal hepatic coma.

The five cases of 'juvenile' cirrhosis (Bearn, Kunkel, and Slater, 1956) had a reduced serum haptoglobin. These patients had active disease with raised serum bilirubin and transaminase levels, and four were subsequently treated with prednisolone as described later.

In idiopathic haemochromatosis the serum haptoglobin level was within the normal range. Although the patients had a definite cirrhosis histologically none showed evidence of liver cell failure and routine liver function tests were normal.

Considering all the cases of cirrhosis together, there was no correlation between the serum albumin and the haptoglobin level ($r = 0{\cdot}113$ $p > 0{\cdot}1$).

2 OBSTRUCTIVE JAUNDICE (INTRAHEPATIC) The patients in this group had all been jaundiced for

some months. The level of serum haptoglobin was very variable and not related to the underlying cause of the cholestasis. In 16 of the 24 cases it was within the normal range (Fig. 1). Seven had an increased concentration. In five patients the serum haptoglobin was over 200 mg./100ml. and two patients with primary biliary cirrhosis had levels of 314 and 324 mg./100ml. respectively. When examined by starch gel electrophoresis those sera with high levels showed a normal haptoglobin pattern. The only patient with a low value had repeated readings of 0 mg./100 ml. There was no evidence of an inhibitory factor in the serum and the absence of serum haptoglobin was confirmed by starch gel electrophoresis. Clinically she had primary biliary cirrhosis and differed in no way from the other patients with normal or raised levels. She probably has a genetically determined anhaptoglobinaemia (Allison, 1959).

Serial readings were obtained in 12 patients with primary biliary cirrhosis. In four of these the serum haptoglobin remained constant. In another four the level of serum haptoglobin increased or decreased with the serum bilirubin level but in the remaining four the level fluctuated for no obvious reason.

Since five of the seven patients with raised serum haptoglobin levels in this group were receiving norethandrolone (Nilevar) therapy for pruritus, the effect of this drug on serum haptoglobin was investigated. Two patients were studied before and during its administration. Their haptoglobin levels rose from 26 to 256 and from 86 to 218 mg./100 ml. and at the same time they became more jaundiced, their serum bilirubin levels rising from 3·0 to 10·6 and from 3·4 to 30 mg./100 ml. respectively; no appreciable changes in serum alkaline phosphatase were recorded.

Extrahepatic The serum haptoglobin was within the normal range with the exception of one patient, who had a raised level of 178 mg./100 ml. This was a young girl with congenital hepatic fibrosis, in whom the bile duct had been damaged at operation. One patient with a bile duct stricture and a level of 138 mg./100 ml. had a previous reading when he was not jaundiced of 62 mg./100 ml.

Considering all the cases of intra- and extrahepatic obstructive jaundice together (whether on or off Nilevar) no correlation was found between the haptoglobin level and the height of the serum bilirubin ($r = 0{\cdot}08$, $P > 0{\cdot}1$). There was, however, a slight though significant correlation ($r = 0{\cdot}360$, $P < 0{\cdot}05$) between the serum concentration of haptoglobin and alkaline phosphatase.

3 INFECTIVE HEPATITIS Serial readings were obtained in seven of the 27 cases and showed a characteristic pattern. Five of these patients had been treated with prednisolone but the same pattern was observed in the other patients who were not given steroids. Fig. 2 shows the course of a patient who had been jaundiced for one week before admission. The initial serum haptoglobin was low but as the serum bilirubin fell the haptoglobin level rose. Subsequently, the level fell again. The inverse relationship between serum bilirubin and haptoglobin during the early recovery phase of hepatitis

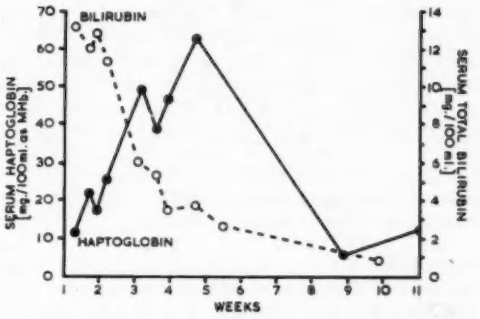

FIG. 2. *Changes in serum bilirubin and haptoglobin levels in a patient with infective hepatitis, who had been jaundiced for one week before admission.*

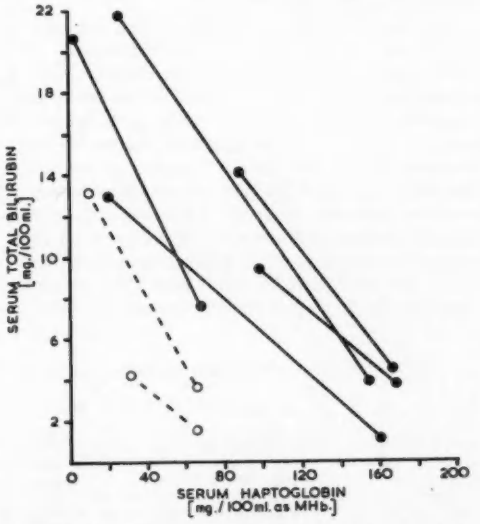

FIG. 3. *The inverse relationship between serum haptoglobin and bilirubin levels during the early recovery phase of infective hepatitis.*
●——● = *prednisolone therapy.*

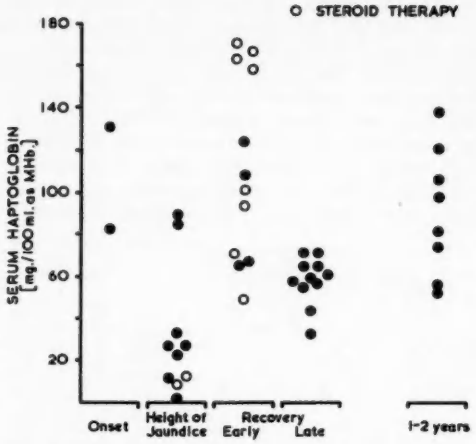

FIG. 4. *Serum haptoglobin levels in 27 cases of infective hepatitis at varying stages of the disease.*

is also shown in Fig. 3. Two cases were seen much earlier in the course of the disease shortly after jaundice had been noticed. The first haptoglobin readings were 128 mg./100 ml. and 84 mg./100 ml. but as jaundice increased during the first week of admission the serum haptoglobin level fell. Subsequently, during recovery the level rose and then fell again as described above.

The results in the remainder of the patients, in whom serial readings were not performed, have been related to the stage of the disease and are shown together with the results in the serial cases (one reading for each stage) in Fig. 4. Although the levels at each stage showed a wide scatter, as might be expected from the different degrees of severity of the cases, the variation between stages is consistent with the sequence described above. It can be seen that the haptoglobin levels during recovery, although within the normal range, are below the normal mean. In a few patients who have been followed subsequently, the level rose slowly during the next few months. This agrees with results from a separate group of patients, who had had hepatitis one to two years previously, and who showed, in general, higher levels distributed around the normal mean. Clinically these patients were well though two had a slightly raised serum bilirubin.

4 CONGENITAL HYPERBILIRUBINAEMIA The serum haptoglobin levels, together with relevant data on these patients, are shown in Table II. Although in none of the cases did the level fall below the lower limit of the normal range (8 mg./100 ml.), in four of the eight patients with Gilbert's disease the serum haptoglobin was 20 mg./100 ml. or less. In two of these the red cell survival was measured and found to be normal whereas in two other patients with Gilbert's disease, who had higher haptoglobin levels, the survival was slightly reduced.

5 MISCELLANEOUS The two patients with congenital hepatic fibrosis had low serum haptoglobin levels (4 and 5 mg./100 ml. respectively). Routine liver function tests in these cases were normal apart from a slightly raised serum alkaline phosphatase. The one patient with a secondary biliary cirrhosis had a level of 7 mg./100 ml. In this patient biliary obstruction had been successfully relieved surgically but had nevertheless progressed to the stage of terminal liver failure. The two patients with cirrhosis with hepatocellular carcinoma were deeply jaundiced and had serum haptoglobin levels of 116 and 204 mg./100 ml.

RELATION WITH INCREASED HAEMOLYSIS

In infective hepatitis no relation has been found between the initial haptoglobin level and the increased rate of haemolysis present, as measured either by Cr^{51} red cell half-life or by the calculated rate of peripheral haemoglobin destruction (Table III). The serum haptoglobin level fluctuates so much during the course of the hepatitis that any such relationship would be unlikely, for the rate of disappearance

TABLE II

SERUM HAPTOGLOBIN CONCENTRATIONS IN 10 CASES OF CONGENITAL HYPERBILIRUBINAEMIA

Case	*Age and Sex*	*Type*	*Haptoglobin (mg./100 ml.)*	*Serum Total Bilirubin (mg./100 ml.)*
1	21 M	Gilbert	9	1·5
2	25 M	Gilbert	12	1·3
3	14 M	Gilbert	12	0·8
4	30 M	Gilbert	20	2·3
5	33 M	Gilbert	30	1·0
6	16 M	Gilbert	41	1·0
7	28 M	Gilbert	58	3·6
8	25 M	Gilbert	81	2·0
9	15 M	Dubin Johnson	38	3·2
10	13 F	Dubin Johnson	71	2·1

of the radioactive cells during this same period does not change (Williams, Pitcher, and Billing 1962).

The calculated peripheral destruction per day depends not only on the rate of cell breakdown, but also on the total amount of circulating haemoglobin and consequently, if this is reduced, as in Case 5 (Table III), who was anaemic, increased haemolysis may not necessarily indicate an increase in the amount of haemoglobin being destroyed. The results show that in cirrhosis and extrahepatic obstruction patients with similar peripheral haemoglobin destruction rates have widely differing haptoglobin levels. A low serum haptoglobin may be found in cirrhosis when there is little increased haemolysis (Case 1) and in obstructive jaundice a high level may occur (Case 9) when there is a marked increase in the rate of haemoglobin destruction. The poor correlation between haptoglobin level and increased red cell breakdown in congenital hyperbilirubinaemia has already been described.

EFFECT OF PREDNISOLONE THERAPY

There is some evidence that giving corticosteroid produces a rise in serum haptoglobin (Brus and Lewis, 1959). The main difficulty in assessing the results in patients with liver disease is therefore to determine which changes are directly due to a non-specific effect of prednisolone and which are secondary to any improvement in liver function that may result. The latter appeared to be the more important factor in the cases of active juvenile cirrhosis treated with prednisolone (Table IV). Three of these showed a definite rise in serum haptoglobin level and in each case liver function also improved with a fall in serum transaminase and serum bilirubin levels. In the fourth case, however, there was no improvement and here the serum haptoglobin remained low.

In obstructive jaundice the problem is simpler, for although giving prednisolone may produce a fall in

TABLE III

SERUM HAPTOGLOBIN LEVEL RELATED TO RATE OF RED CELL BREAKDOWN AND PERIPHERAL HAEMOGLOBIN DESTRUCTION

Case	*Diagnosis*	*Serum Total Bilirubin (mg./100 ml.)*	*Haemoglobin (g./100 ml.)*	*Cr^{51} Red Cell Half-life (days)*	*Haemoglobin Destruction (mg./kg./day)*	*Serum Haptoglobin (mg./100 ml.)*
1	Cirrhosis	1·1	11·8	25	259	4
2	Cirrhosis	1·6	14·1	24	350	11
3	Cirrhosis	6·3	10·1	18	426	3
4	Cirrhosis	7·4	10·0	13	453	11
5	Intrahepatic obstructive jaundice	20·2	9·6	14	278	416
6	Intrahepatic obstructive jaundice	13·0	11·9	21	363	41
7	Intrahepatic obstructive jaundice	15·3	12·4	18	407	84
8	Intrahepatic obstructive jaundice	40·8	7·7	13	447	162
9	Extrahepatic obstructive jaundice	31·0	12·2	12	527	178
10	Infective hepatitis	6·8	14·3	22	270	129
11	Infective hepatitis	13·9	10·1	17	285	23
12	Infective hepatitis	19·6	11·2	14	304	89
13	Infective hepatitis	13·1	13·9	23	375	10
1[1]	Congenital hyperbilirubinaemia	1·5	13·9	25	338	9
4[1]	Congenital hyperbilirubinaemia	2·3	15·5	25	270	20
5[1]	Congenital hyperbilirubinaemia	1·1	15·4	21	357	30
6[1]	Congenital hyperbilirubinaemia	1·0	15·1	18	410	41

[1]These are the same case numbers as in Table II.

TABLE IV

SERUM HAPTOGLOBIN AND BILIRUBIN LEVELS IN FOUR CASES OF ACTIVE 'JUVENILE' CIRRHOSIS BEFORE AND AFTER PREDNISOLONE THERAPY

Case	*Serum Total Bilirubin (mg./100 ml.)*		*Serum Haptoglobin (mg./100 ml.)*		*Duration of Therapy*
	Before	*After*	*Before*	*After*	
1	3·0	1·0	3	23	4 months
2	8·0	1·0	7	49	4 months
3	4·5	2·0	8	43	3 weeks
4	6·3	12·3	3	4	5 weeks

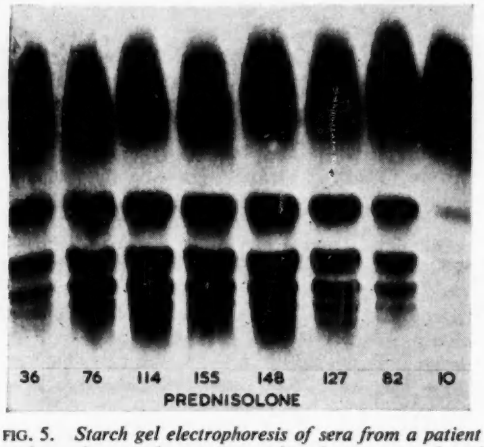

FIG. 5. *Starch gel electrophoresis of sera from a patient with intrahepatic obstructive jaundice showing an unaltered haptoglobin pattern despite marked variation in level with prednisolone therapy.*

serum bilirubin, this is not very marked and the effect is purely temporary. Prednisolone was given to five of the patients with chronic intrahepatic obstruction. In one, the haptoglobin level was already falling and continued to do so despite prednisolone therapy. Two others showed a slight rise in serum haptoglobin coincident with a slight fall in serum bilirubin, these changes being reversed when treatment was discontinued. The remaining two showed a marked and rapid rise in serum haptoglobin (36 to 155 mg./100 ml. over eight days, and 41 to 102 mg./100 ml. over two weeks respectively), whereas the serum bilirubin fell only slightly in the first and rose steadily in the second patient throughout the period of observation. Subsequently there was an equally rapid fall, though steroid therapy was continued in one patient. The serum of one of these was subjected to starch gel electrophoresis and it was found that the haptoglobin pattern remained unaltered in spite of changes in serum concentration ranging from 10 to 155 mg./100 ml. (Fig. 5). It seems very likely that the response in these two patients was simply a non-specific effect of prednisolone on haptoglobin level.

Three cases of extrahepatic obstructive jaundice had steroid therapy. Two showed no change while the third had a slight rise in serum haptoglobin together with a slight fall in serum bilirubin, the response being similar to that seen in some of the cases of intrahepatic obstruction described above.

The effect of steroid therapy in infective hepatitis is also difficult to assess. There the serum haptoglobin rises markedly as the serum bilirubin begins to fall, this occurring with and without prednisolone therapy. The extent of the rise, however, is probably influenced by prednisolone as suggested by Fig. 3, although this is difficult to assess since the two groups were not of comparable severity and the higher levels reached in the treated cases may simply reflect the greater severity of disease.

DISCUSSION

Seventy per cent of the 73 patients with cirrhosis and obstructive jaundice had values falling within the normal range so that an isolated haptoglobin estimation is of limited value in the diagnosis of these conditions. Nevertheless, certain trends are apparent and the finding of an abnormally low level in the presence of liver disease is very suggestive of cirrhosis. This may be of considerable diagnostic help in the well-compensated patient, who shows few clinical signs. Nevertheless, there are many exceptions and the association in some patients of frank, decompensated liver disease with normal levels is unexplained. In obstructive jaundice the estimation seems of less value, for with one exception patients with extrahepatic obstruction had levels within the normal range. This is contrary to the findings of Owen *et al.* (1961b) who reported that 81% of their patients showed values over 150 mg./100 ml. However, our series is mainly composed of patients with carcinomatous obstructions whereas the majority of Owen's patients had obstruction due to gall stones. Nyman (1959) also found that it was only in the latter type of case, in which cholangitis is present, that the level was raised. The serum haptoglobin is of little value in distinguishing between intra- and extrahepatic obstructive jaundice, a point which has not been stressed before. It may, however, be of considerable value in the sometimes difficult differentiation between infective hepatitis and obstructive jaundice. A falling haptoglobin level in the presence of rapidly increasing jaundice is diagnostic of hepatitis (Owen *et al.*, 1961b).

The mechanism of these changes in liver disease is uncertain. Owen and his colleagues (1959) found some correlation between the haptoglobin level and the serum albumin suggesting that the low levels were related to impaired hepatic synthesis of protein. This was not found by Nyman (1959) and in the present series there was no correlation with serum albumin. There does, however, appear to be a relation with the activity of the disease. The patients with active juvenile cirrhosis in whom the haptoglobin level rose with steroid therapy also showed improvement in liver function. Also, the majority of the decompensated cirrhotics had low levels whereas the patients with alcoholic cirrhosis or haemo-

chromatosis, who had a quiescent type of lesion, had a serum haptoglobin within the normal range. Low levels, however, were found in two cases of congenital hepatic fibrosis, a condition in which there is little disturbance of liver function. In any case, there is as yet no direct evidence that the liver is the source of haptoglobin and the dramatic rises observed following infection and surgery have suggested a possible origin in connective tissue (Jayle and Boussier, 1955).

It is clear also that the low serum haptoglobin in cirrhosis cannot be attributed to haemolysis. Indeed Williams *et al.* (1962) have shown that increased haemolysis is common to all forms of jaundice, including that due to biliary obstruction, in which the haptoglobin level may be normal or high. Another possible explanation is altered oestrogen metabolism. Administration of these steroids has been shown to lower the serum haptoglobin (Borglin and Nyman, 1961) and it is known that their conjugation and excretion in liver disease is sometimes impaired (Cameron, 1957).

There was a slight, though significant, correlation between the level of serum haptoglobin and alkaline phosphatase. This suggests that the higher level in obstruction might be related to biliary retention. This presumes that haptoglobin is normally excreted in bile but in preliminary experiments we have only been able to demonstrate the presence of haptoglobin in bile in one case and this was a patient with an infection, who had a serum level of over 500 mg./100 ml. If biliary retention were a factor one would also expect a correlation with serum bilirubin which was not found. It is germane to consider here the effect of norethandrolone (Nilevar) therapy, a known cause of intrahepatic cholestasis. Cases of primary biliary cirrhosis receiving norethandrolone did tend to have higher serum haptoglobin values and the administration of this drug to two early cases caused a definite rise in level. This was accompanied by a rise in serum bilirubin although the serum alkaline phosphatase was unaltered. We cannot, therefore, be sure whether the rise in haptoglobin with norethandrolone is a secondary result of increased biliary retention or whether this drug has a non-specific effect similar to that of prednisolone. The abrupt and temporary rises in serum haptoglobin noted in some of the cases of intrahepatic obstruction are almost certainly due to a non-specific effect of prednisolone.

The alterations in serum haptoglobin in infective hepatitis are of interest. The fall in level as jaundice increases and the sudden rise with the onset of recovery are difficult to explain. If the rise were due to improvement in liver function then it is surprising that the level subsequently falls. Although the extent of the rise was greater in the patients on prednisolone therapy these were the most severe cases, and a definite increase occurred at this stage even in the patients who did not receive corticosteroids. Relatively few of these cases had serial estimations; Nyman (1959) has, however, reported a similar pattern in some of her cases of sporadic hepatitis.

In conclusion it is clear that there is as yet no satisfactory explanation for the changes in serum haptoglobin observed in differing types of liver disease. However, it is possible that turnover studies using haptoglobin labelled with radioactive iodine may help to elucidate this problem.

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REFERENCES

Allison, A. C. (1959). Genetic control of human haptoglobin synthesis. *Nature (Lond.)*, **183**, 1312-1314.

Bearn, A. G., Kunkel, H. G., and Slater, R. J. (1956). The problem of chronic liver disease in young women. *Amer. J. Med.*, **21**, 3-15.

Borglin, N. E., and Nyman, M. (1961). The effect of estrogens on the haptoglobin level in the blood. *Scand. J. clin. Lab. Invest.*, **13**, 107-115.

Brus, I., and Lewis, S. M. (1959). The haptoglobin content of serum in haemolytic anaemia. *Brit. J. Haemat.*, **5**, 348-355.

Cameron, C. B. (1957). The liver and steroid hormone metabolism. *Brit. med. Bull.*, **13**, 119-125.

Jayle, M. F., and Boussier, G. (1955). Les séromucoïdes du sang, leurs relations avec les mucoprotéines de la substance fondamentale du tissu conjonctif. *Expos. ann. Biochim. méd.*, **17**, 157-194.

——, ——, and Tonnelat, J. (1956). Isolement à partir du sérum humain de la combinaison hémoglobine—haptoglobine à l'état homogène. *Bull. Soc. Chim. biol. (Paris)*, **38**, 343-349.

——, and Vallin, J. (1952). Variations de la formule protéique du plasma au cours des affections hépatiques. *Sem. Hôp. Paris*, **28**, 3133-3138.

King, E. J., and Wootton, I. D. P. (1959). *Micro-analysis in Medical Biochemistry*, 3rd ed. (reprint), pp. 82-84. Churchill, London.

Malloy, H. T., and Evelyn, K. A. (1937). The determination of bilirubin with the photoelectric colorimeter. *J. biol. Chem.*, **119**, 481-490.

Nyman, M. (1959). Serum haptoglobin: methodological and clinical studies. *Scand. J. clin. Lab. Invest.*, **11**, Suppl. 39.

Owen, J. A., Better, F. C., and Hoban, J. (1960). A simple method for the determination of serum haptoglobins. *J. clin. Path.*, **13**, 163-164.

——, Gruchy, G. C. de, and Smith, H. (1960). Serum haptoglobins in haemolytic states. *Ibid.*, **13**, 478-482.

——, Mackay, I. R., and Got, C. (1959). Serum haptoglobins in hepatobiliary disease. *Brit. med. J.*, **1**, 1454-1457.

——, Podanyi, R., and Smith, H. (1961a). The effect of surgery on serum haptoglobins. *Proc. Ass. Clin. Biochem.*, **1**, 89-91.

——, ——, —— (1961b). Serum haptoglobins and other tests in the diagnosis of hepatobiliary jaundice. *Clin. Sci.*, **21**, 189-197.

Smith, H., and Owen, J. A. (1961). The determination of haptoglobins in normal human serum. *Biochem. J.*, **78**, 723-728.

Smithies, O. (1955). Zone electrophoresis in starch gels: Group variations in the serum proteins of normal human adults. *Ibid.*, **61**, 629-641.

Veall, N., and Vetter, H. (1958). *Radioisotope Techniques in Clinical Research and Diagnosis*. Butterworth, London.

Williams, R., Pitcher, C. S., and Billing, B. H. (1962). Red cell survival in infective hepatitis and other forms of jaundice. In preparation.

Gut, 1961, **2**, 304

Plasma free fatty acid in liver disease

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SYNOPSIS The plasma free fatty acid level was raised in the majority of 131 patients with a variety of liver disorders, and included both those with severe liver cell damage and those with relatively good liver cell function and obstructive jaundice. This raised level is probably due to an increased mobilization of depot fat, for glucose, which inhibits peripheral liberation of free fatty acids, caused a fall in the plasma level and obliteration of the femoral venous-arterial difference in patients with liver disease comparable with that in normal persons. In addition, the plasma free fatty acid flux was measured using palmitate-l-C^{14} and was found to be increased in patients with liver disease.

Free or non-esterified fatty acids have only recently been accurately determined in plasma (Davis, 1947; Dole, 1956; Gordon, 1957). Formerly, such acids were often considered to be artefacts of the analytical technique, for they represent only a small fraction (less than 5%) of the total fatty acids of the plasma. They provide a major source of energy for the body (Frederickson and Gordon, 1958b). The source of the plasma free fatty acids is mainly adipose tissue where fatty acids are stored in the esterified form as glycerides, and their liberation from adipose tissue into the blood, where they are mainly bound to albumin, is modified by various factors. Broadly speaking, an increased release of free fatty acids, which causes a high plasma level, occurs when the body suddenly needs energy, *e.g.*, in conditions of stress (Dole, 1956; Bogdonoff, Estes, and Trout, 1959), or when utilization of carbohydrate is impaired or decreased, *e.g.*, in uncontrolled diabetes or prolonged starvation (Laurell, 1956; Bierman, Dole, and Roberts, 1957; Albrink and Neuwirth, 1960). Conversely when glucose is given as an alternative source of energy, the liberation of fatty acid is inhibited and the plasma level of free fatty acids falls (Dole, 1956).

Patients with liver disease have an abnormal intermediate carbohydrate metabolism (Amatuzio and Nesbitt, 1950; Dawson, de Groote, Rosenthal, and Sherlock, 1957), and so might theoretically use fat as a major alternative source of energy. Indeed, recently Wajchenberg, Hoxter, Mello, and Ulhoa Cintra (1960) found a raised plasma level of free fatty acids in 18 patients with liver disease.

This paper reports the plasma free fatty acid levels in a series of 131 patients with a variety of liver disorders. In an attempt to clarify the nature of the disordered metabolism of free fatty acids in liver disease, some observations were made on the effect of parenteral glucose on the plasma level of free fatty acids and its flux was estimated using palmitate-l-C^{14}.

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PATIENTS STUDIED AND METHODS

One hundred and thirty-one patients with liver disease were studied. Twenty-six had alcoholic cirrhosis, 65 had non-alcoholic cirrhosis, of whom 12 were of the juvenile type (Bearn, Kunkel, and Slater, 1956), 14 had primary biliary cirrhosis, four had secondary biliary cirrhosis, nine had extrahepatic biliary obstruction (seven due to a new growth and two to gallstones), five had haemochromatosis but did not require insulin, and eight had viral hepatitis. The only patients who had an associated disorder known to affect metabolism of free fatty acids were those in whom new growth was causing obstructive jaundice (Mueller and Watkin, 1961). The 20 normal control subjects were either medical staff or students.

All subjects had fasted overnight and were resting; the 11 patients attending the out-patient clinic were allowed to rest on a couch for one hour before the blood was taken from an antecubital vein. This was put into a heparin tube and the plasma was separated by centrifuging at 0°C., usually within 15 minutes but occasionally up to one hour after the blood was taken. The separated plasma was either analysed immediately or after storage for less than four hours in the deep freeze. Dole's method (1956) was used for the free fatty acid determination but Nile blue instead of thymol blue was used as the indicator. The coefficient of variance of 24 determinations on a single plasma was 2·2%. Recovery of palmitic acid added to plasma was 95%.

In 10 patients with hepatic cirrhosis and six normal

subjects, arterial free fatty acid levels were measured at two 15-minute intervals before, and four 30-minute intervals after, an intravenous injection of glucose (0·5 g. per kg. body weight). In nine subjects the femoral vein and in seven subjects the antecubital vein free fatty acids were measured simultaneously with the arterial level. Both arterial and venous blood was obtained from an indwelling polythene catheter; heparin was not used to keep the catheter patent.

The fractional turnover rate and flux of plasma free fatty acids were estimated using palmitate-1-C^{14} (Laurell, 1957; Fredrickson and Gordon, 1958a). This was obtained from the Radiochemical Centre, Amersham, and dissolved in a 0·5% human albumin solution in physiological saline. The solution was sterilized by passing through a Seitz filter, and the final concentration was approximately 10μc. per ml. In eight fasting patients with hepatic cirrhosis who did not have fluid retention and in two subjects without liver disease, 1 ml. of the albumin-palmitate-1-C^{14} solution was given intravenously and rapid timed samples of blood were obtained from an indwelling polythene arterial catheter during the 11 minutes following the injection. The arterial plasma free fatty acid level was measured chemically at the beginning and end of the procedure and did not alter significantly. All samples were extracted in duplicate by Dole's method, and the free fatty acid in the heptane phase was removed from the other lipid by washing with 50% alkaline ethanol (Borgström, 1952). The radioactive free fatty acid in the alkaline ethanol was estimated by adding 1 ml. aliquots to 10 ml. of dioxane which contained a liquid phosphor (Davidson, 1958). It was then counted in an Echo liquid scintillation counter. The plasma volume was measured using I^{131} labelled-albumin, except in three patients with cirrhosis and in one with no liver disease in whom it was estimated according to the method of Caesar, Shaldon, Chiandussi, Guevara, and Sherlock (1961).

FASTING PLASMA FREE FATTY ACID IN LIVER DISEASE

The normal fasting venous free fatty acid level was 0·52 ± 0·14 mEq./l. (mean ± 2SD) (Fig. 1). The fasting venous levels were above the normal range on 135 of 146 occasions in 131 patients with liver disease (Fig. 1). A similar proportion of high values was observed in all types of liver disease. It made no difference whether the underlying disease affected mainly liver cells, as in cirrhosis and hepatitis, or whether it was associated with jaundice and good liver cell function as in primary biliary cirrhosis. In nine patients with stable liver function, serial readings over a two- to eight-week period were remarkably constant (Fig. 2).

There was no correlation between the plasma free fatty acid level and the serum albumin in 38 randomly-selected patients with cirrhosis (Fig. 3). This is true whether one considers the patients as a

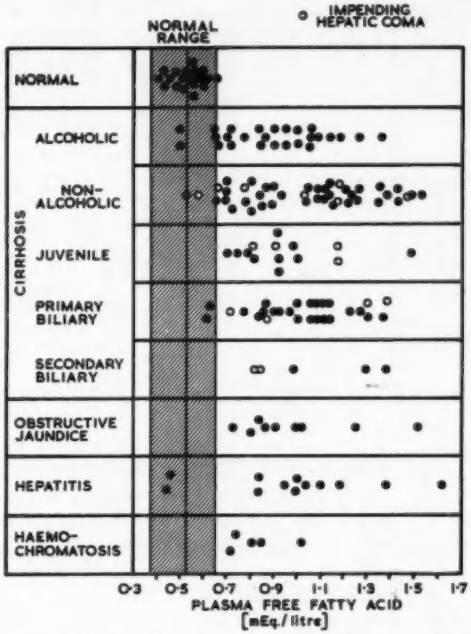

FIG. 1. *Fasting plasma free fatty acid levels in patients with liver disease.*
The 146 readings were obtained from 131 patients. The mean ± 2SD of the normal controls is shown by the shaded area.

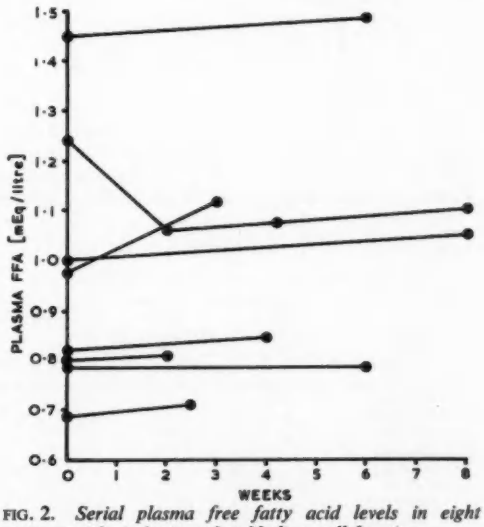

FIG. 2. *Serial plasma free fatty acid levels in eight patients with cirrhosis and stable liver cell function.*

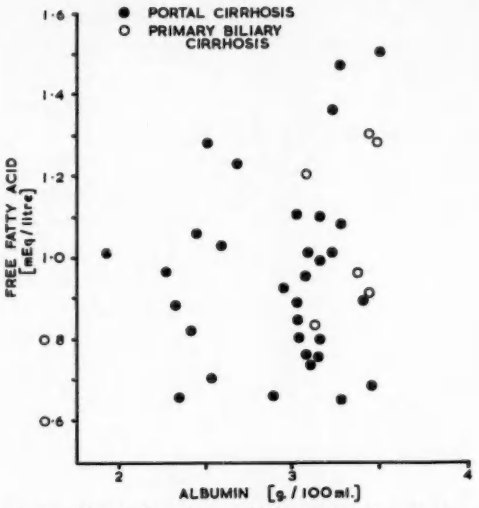

FIG. 3. *Plasma free fatty acid level plotted against the serum albumin in 38 patients with cirrhosis.*

single group or separates the patients with primary biliary cirrhosis. Similarly, there was no correlation between plasma free fatty acid and the serum bilirubin levels, which varied from 1·2 to 35 mg. per 100 ml. Patients with impending hepatic coma did not have a higher plasma level than those who were mentally alert (Fig. 1).

ORIGIN OF RAISED PLASMA FREE FATTY ACIDS IN LIVER DISEASE

LIBERATION OF PLASMA FREE FATTY ACIDS IN VITRO

The raised plasma free fatty acid levels might have been artefacts due to their liberation *in vitro* between the time of sampling and analysis. Such hydrolysis of blood *in vitro* occurs after an injection of heparin (Rizack, 1960) due to the liberation of lipoprotein lipase into the plasma, and recently Baker, Levine, Turner, and Dubin (1958) found a high liproprotein lipase level in the blood of patients with cirrhosis. To exclude this possibility, on 10 occasions the analysis was performed at 15 minutes and 60 minutes after venepuncture. Free fatty acids were not liberated to a significant extent during this time when the blood had been standing at room temperature (Table I).

TABLE I

EFFECT OF STANDING ON PLASMA FREE FATTY ACID LEVEL *IN VITRO*

Diagnosis	*Time after Shedding Blood*	
	15 min. (mEq/l.)	*60 min. (mEq/l.)*
Cirrhosis	1·17	1·13
Cirrhosis	1·13	1·15
Cirrhosis	1·06	1·10
Cirrhosis	0·99	0·98
Cirrhosis	0·88	0·90
Control	0·66	0·66
Control	0·62	0·64
Control	0·59	0·58
Control	0·56	0·59

THE EFFECT OF INTRAVENOUS GLUCOSE ON PLASMA FREE FATTY ACIDS

After intravenous glucose, the lowest arterial level in five normal subjects was between 0·25 and 0·4 mEq./l. (Fig. 4). The plasma level of eight of the 10 patients with cirrhosis fell to the same range. The

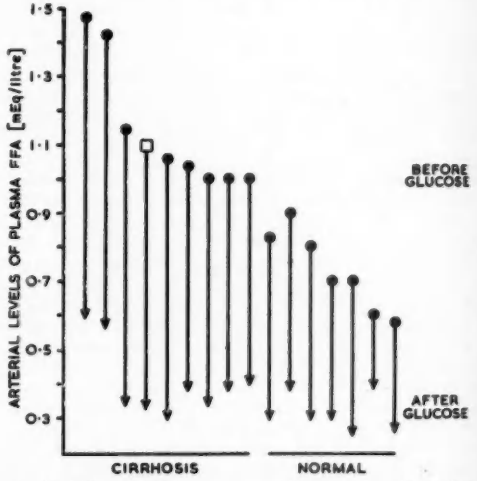

FIG. 4. *The effect of intravenous glucose on arterial free fatty acid levels in 10 patients with cirrhosis and six normal subjects.* □ *Represents a patient with primary biliary cirrhosis.*

two patients with the highest fasting level failed to reach this range although they had a greater absolute fall than the normal persons. The slightly raised fasting level in two of the normal subjects was probably due to the stress of arterial puncture (Cardon and Gordon, 1959).

The resting femoral vein arterial free fatty acid difference varied from 0·17 to 0·3 mEq./l., and the values for the three normal subjects were similar to those of the six patients with liver disease (Fig. 5). After intravenous glucose, the femoral venous-arterial difference contracted markedly to between

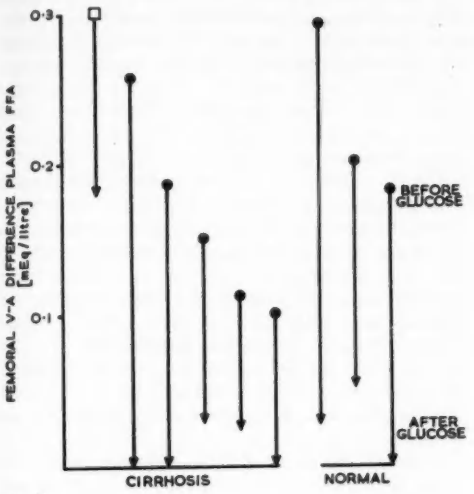

FIG. 5

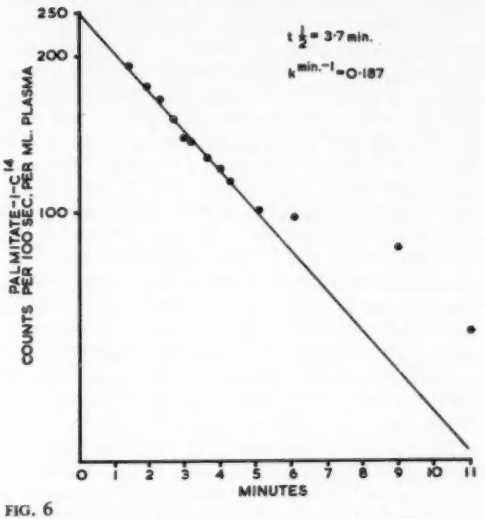

FIG. 6

FIG. 5. *The effect of intravenous glucose on the femoral vein-arterial free fatty acid difference in six patients with cirrhosis and three normal subjects.* □ *Represents a patient with primary biliary cirrhosis.*

FIG. 6. *The disappearance of palmitate-l-C*^{14} *from the plasma in a patient with cirrhosis, fasted for 12 hours.*

0 and 0·05 mEq./l. in the three normal persons and in the five patients with portal cirrhosis; the patient with primary biliary cirrhosis had a less impressive contraction to 0·18 mEq./litre.

The fasting antecubital venous-arterial difference was minimal, being less than 0·01 mEq./l. in three normal persons and in four patients with liver diseases: this might be expected, for the antecubital vein drains the forearm, which is mainly lean tissue.

PALMITATE-L-C^{14} TURNOVER

After injection there is a rapid exponential fall in concentration of plasma palmitate-l-C^{14} for the first two to five minutes; the disappearance then becomes more complex. The half-time of this initial fall was measured, and from this the rate constant ($K = 0{\cdot}693/t_{\frac{1}{2}}$) was calculated and represents the fraction of the plasma free fatty acids which dis-

TABLE II

PLASMA FREE FATTY ACID FLUX IN LIVER DISEASE

Diagnosis	*Weight (kg.)*	*T½ (min.)*	*K (min.⁻¹)*	*Total Plasma Volume (ml.)*	*Plasma FFA (mEq./l.)*	*Total Circulating FFA (mEq.)*	*Plasma FFA Flux (mEq./min.)*	*Plasma FFA Flux per Kg. Body Weight (mEq./min./kg.) (× 10⁻⁴)*
Cirrhosis	69	3·7	0·19	3,919	1·100	4·311	0·81	117
Cirrhosis	76	1·95	0·35	3,952[1]	1·060	4·189	1·46	193
Cirrhosis	57·5	2·45	0·28	3,500	1·300	4·550	1·27	221
Cirrhosis	65·6	2·55	0·27	3,411[1]	0·950	3·240	0·87	134
Cirrhosis	64	2·50	0·28	4,440	1·160	5·150	1·44	225
Cirrhosis	47	3·8	0·18	3,640	1·333	4·852	0·88	187
Cirrhosis	41·8	2·1	0·33	2,176[1]	1·082	2·354	0·77	185
Cirrhosis	64·5	2·1	0·33	3,590	1·155	4·146	1·37	213
Mean	60·9	2·6	0·27	3,578	1·142	4·099	1·11	184
Control	81	2·34	0·30	3,760	0·680	2·556	0·77	95
Control	83·7	1·9	0·36	3,385[1]	0·600	2·031	0·73	87
Mean	82·4	2·1	0·33	3,572	0·640	2·293	0·75	92

[1]Calculated according to Caesar *et al.* (1961).

appears each minute. When K is multiplied by the total plasma free fatty acid, which is obtained by multiplying the plasma volume by the plasma free fatty acid, the result equals the flux or the amount of plasma free fatty acid disappearing each minute. The results of such estimations in eight patients with portal cirrhosis and in two normal persons are shown in Table II. The mean plasma flux was 0·75 mEq./min. for the two persons without liver disease. This is comparable to the estimation of Fredrickson and Gordon (1958a) who calculated a mean flux of 0·66 mEq./min. in 18 normal persons after an overnight fast. The eight patients with cirrhosis all had a high plasma free fatty acid flux (0·81 to 1·44 mEq./min.) with a mean of 1·06 mEq./min. The difference between the two groups is even more impressive if a correction is made for body weight, as the patients with cirrhosis were not as heavy as the controls (Table II).

As an excess of adrenaline is a common cause of a raised plasma free fatty acid level, the urinary excretion of the main metabolite of adrenaline, 3 methoxy 4 hydroxymandelic acid (VMA), was measured in two patients with portal cirrhosis and two with primary biliary cirrhosis who had a plasma free fatty acid level of over 1 mEq./litre. This was normal.

DISCUSSION

In this study, the normal range of fasting antecubital venous free fatty acid measured by Dole's method was similar to that previously reported (Munkner, 1959; Wajchenberg *et al.*, 1960; Corvilain, Loeb, Champenois, and Abramow, 1961). The almost invariably raised plasma free fatty acid level in patients with all types of liver disease amplifies the observations of Wajchenberg *et al.* (1960) who found such raised plasma levels in 18 patients with hepatocellular disease. In the present series patients with obstructive jaundice and minimal hepatocellular damage also had a high level, although in some of these patients the underlying neoplastic disease might have contributed to it (Mueller and Watkin, 1961). It was surprising that there was no apparent correlation between plasma free fatty acid levels and the degree of liver cell failure, the depth of jaundice or the presence of neuropsychiatric complications. It might be that serial observations on a single patient with a fluctuating clinical course would be more valuable than comparing groups of patients with different clinical states. Our serial observations were on patients with stable liver function, and the plasma free fatty acid levels were remarkably steady. These observations differ from those of Stormont and Mackie (1959) who found a raised plasma free fatty acid level mainly in patients with severe hepatocellular failure, especially when hepatic coma was present. These differences might be explained by the fact that their normal range was much higher than ours (0·76 $\pm$ 0·04 mEq./l., mean $\pm$ SE) and that most of their patients probably, in addition to liver disease, had metabolic disorders associated with alcoholism.

Dole's method partially estimates short chain organic acids in addition to plasma free fatty acids. These include pyruvic acid and α ketoglutamic acid, which are raised in the plasma of patients with liver disease (Dawson *et al.*, 1957). The results of the intravenous glucose test discount the suggestion that the raised levels are due to an increase in short chain acids, for after glucose the plasma level of such acids rise or remain constant (Amatuzio, Shrifter, Stutzman, and Nesbitt, 1952) while the true plasma free fatty acid level falls, as was observed in our patients.

A raised plasma level may, theoretically, be due to an increased mobilization of peripheral fat, to diminished utilization of free fatty acids, or to hydrolysis of esterified fat in the blood stream. Hydrolysis of esterified fat in blood did not continue *in vitro* after the blood was drawn, and this cause is unlikely. Raised plasma levels in various clinical situations such as prolonged starvation, diabetes, thyrotoxicosis, and 'stress' are thought to be mainly due to increased mobilization of tissue fat, though often the evidence for this has been indirect. If the high plasma free fatty acid level were due to decreased utilization, suppression of the peripheral release of free fatty acids would be followed by a less impressive fall in the arterial level in the affected group than in the controls. Conversely, if the raised levels were due to an increased mobilization of tissue fat, an inhibition of this process would be followed by a rapid fall in arterial free fatty acid levels which would be equal to or greater than the controls. Glucose inhibits the release of free fatty acids from adipose tissue both *in vitro* (Raben and Hollenberg, 1960) and *in vivo* as shown by the obliteration of the femoral venous-arterial free fatty acid difference (Gordon, 1957). The similar response to intravenous glucose of our patients and normal persons, both with respect to the obliteration of the femoral venous-arterial difference and the associated fall of plasma free fatty acids, suggests that the increased level is not due to impaired utilization of plasma free fatty acids. More direct evidence for the cause of the raised plasma level is given by the palmitate-l-C^{14} studies. During these studies the plasma free fatty acid levels remained constant and so the increased flux of palmitate-l-C^{14} in patients with liver disease means an increased production as well as removal of

plasma free fatty acids. The assumptions in such calculations have been discussed by Laurell (1957) and by Frederickson and Gordon (1958a). The calculated plasma free fatty acid flux in these patients was comparable to that observed in a patient after prolonged starvation (Laurell, 1957) when the body is mainly dependent upon fat as a source of energy.

Not all free fatty acids which are removed from the plasma are immediately oxidized, and the balance is re-esterified in the liver before being transported by the blood back to peripheral tissues for storage (Frederickson and Gordon, 1958b). When excessive, this may contribute to the formation of a fatty liver and a raised blood glyceride level. Such a raised level has only been reported in obstructive jaundice (Phillips, 1960) and not in portal cirrhosis (Wajchenberg *et al.*, 1960). The mechanism for the increased mobilization of tissue fat remains undetermined. It seems unlikely that adrenaline, which so frequently causes this phenomenon, can be implemented, for the urinary excretion of its main metabolite (VMA) was normal in four patients with a liver disorder and raised plasma levels of free fatty acids.

We wish to thank Professor J. Caroli, who allowed us to study some of his patients with alcoholic cirrhosis, and also Dr. Merton Sandler, who performed the V.M.A. estimations.

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REFERENCES

Albrink, M. J., and Neuwirth, R. S. (1960). Effect of previous starvation on the response of plasma lipids and free fatty acids to a fat meal. *J. clin. Invest.*, **39**, 441-446.

Amatuzio, D. S., and Nesbitt, S. (1950). A study of pyruvic acid in the blood and spinal fluid of patients with liver disease with and without hepatic coma. *Ibid.*, **29**, 1486-1490.

——, Shrifter, N., Stutzman, F. L., and Nesbitt, S. (1952). Blood pyruvic acid response to intravenous glucose or insulin in the normal and in patients with liver disease and with diabetes mellitus. *Ibid.*, **31**, 751-755.

Baker, S. P., Levine, H., Turner, L., and Dubin, A. (1958). Lipoprotein lipase response in Laennec's cirrhosis. *Proc. Soc. exp. Biol.* (*New York*), **99**, 670-672.

Bearn, A. G., Kunkel, H. G., and Slater, R. J. (1956). The problem of chronic liver disease in young women. *Amer. J. Med.*, **21**, 3-15.

Bierman, E. L., Dole, V. P., and Roberts, T. N. (1957). An abnormality of nonesterified fatty acid metabolism in diabetes mellitus. *Diabetes*, **6**, 475-479.

Bogdonoff, M. D., Estes, E. H., and Trout, D. (1959). Acute effect of psychological stimuli upon plasma non-esterified fatty acid level. *Proc. Soc. exp. Biol.* (*New York*), **100**, 503-504.

Borgström, B. (1952). Investigation on lipid separation methods. *Acta physiol scand.*, **25**, 111-119.

Caesar, J., Shaldon, S., Chiandussi, L., Guevara, L., and Sherlock, S. (1961). The use of indocyanine green in the measurement of hepatic blood flow and as a test of hepatic function. *Clin. Sci*, **21**, 43-57.

Cardon, P. V., and Gordon, R. S. (1959). Rapid increase of plasma unesterified fatty acids in man during fear. *J. Psychosom. Res.* **4**, 5-9.

Corvilain, J., Loeb, H., Champenois, A., and Abramow, M. (1961). Effect of fasting on levels of plasma-nonesterified fatty acids in normal children, normal adults, and obese adults. *Lancet*, **1**, 534-535.

Davidson, J. D. (1958). In *Liquid Scintillation Counting*, pp. 88-95. Eds. Bell, C. G. and Hayes, F. Newton. Pergamon Press, London.

Davis, B. D. (1947). The estimation of small amounts of fatty acid in the presence of polyoxyethylene sorbitan partial fatty acid esters ("Tween") and of serum proteins. *Arch. Biochem.*, **15**, 351-358.

Dawson, A. M., de Groote, J., Rosenthal, W. S., and Sherlock, S. (1957). Blood pyruvic-acid and alpha-ketoglutaric-acid levels in liver disease and hepatic coma. *Lancet*, **1**, 392-396.

Dole, V. P. (1956). A relation between non-esterified fatty acids in plasma and the metabolism of glucose. *J. clin. Invest.*, **35**, 150-154.

Fredrickson, D. S., and Gordon, R. S. (1958a). The metabolism of albumin-bound C^{14}-labelled unesterified fatty acids in normal human subjects. *Physiol Rev.*, **37**, 1504-1515.

——, —— (1958b). Transport of fatty acids. *Ibid.*, **38**, 585-630.

Gordon, R. S. (1957). Unesterified fatty acid in human blood plasma. II. The transport function of unesterified fatty acid. *J. clin. Invest.*, **36**, 810-815.

Laurell, S. (1956). Plasma free fatty acids in diabetic acidosis and starvation. *Scand. J. clin. Lab. Invest.*, **8**, 81-82.

—— (1957). Turnover rate of unesterified fatty acids in human plasma. *Acta Physiol. scand.*, **41**, 158-167.

Mueller, P. S., and Watkin, D. M. (1961). Plasma unesterified fatty acid concentrations in neoplastic disease. *J. Lab. clin. Med.*, **57**, 95-108.

Munkner, C. (1959). Fasting concentrations of non-esterified fatty acids in diabetic and non-diabetic plasma and diurnal variations in normal subjects. *Scand. J. Lab. clin. Invest.*, **11**, 388-393.

Phillips, G. B. (1960). The lipid composition of serum in patients with liver disease. *J. clin. Invest.*, **39**, 1639-1650.

Raben, M. S., and Hollenberg, C. H. (1960). Effect of glucose and insulin on the esterification of fatty acids by isolated adipose tissue. *Ibid.*, **39**, 435-439.

Rizack, M. A. (1960). Intravascular effect of heparin on plasma nonesterified fatty acid and triglyceride during alimentary lipemia. *Proc. Soc. exp. Biol.* (*New York*), **104**, 111-113.

Stormont, J. M., and Mackie, J. E. (1959). An abnormality of non-esterified fatty acid metabolism in alcoholic patients with cirrhosis. *J. clin. Invest.*, **38**, 1047 (Abs.).

Wajchenberg, B. L., Hoxter, G., Mello, E. H. L., and Ulhoa Cintra, A. B. (1960). Non-esterified and esterified fatty acids in hepatocellular disease. *Lancet*, **1**, 1218-1219.

Gut, 1961, 2, 310

Changes in the portal and splenic veins in portal hypertension and their relation to splenomegaly[1]

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SYNOPSIS This paper is a pathological study on the portal veins demonstrating that the changes in the portal circulation and in the spleen reflect the degree or duration of portal hypertension.

Cirrhosis of the liver has been known for many years to be accompanied by changes in the portal venous system and the spleen, and they were thought to be secondary to the congestion brought about by the distortion of the lobular pattern in the liver; somewhat similar changes occur in the absence of cirrhosis of the liver when the portal or splenic veins are blocked. The congestion gives rise to an increase in pressure in the vein (portal hypertension) and the development of a collateral circulation. Banti (1898) described the changes in the portal vein as a phlebosclerosis, and later Pei-Lin Li (1940), studying the pathological changes in the vein wall in greater detail, considered that the medial hypertrophy and intimal thickening were due to an increase in pressure in the vein.

In the hope that examination of the portal and splenic veins would give some indication as to the extent or duration of the congestion, portions of these veins were obtained from cases with cirrhosis of the liver and portal congestion with splenomegaly, then carefully studied. The pathological changes in them were noted and correlated with the other lesions, including the size of the spleen. A liver was taken to be cirrhotic if the following changes were seen: 1, Obvious nodularity, 2, an excess of fibrous tissue throughout, and 3, marked distortion of the lobular architecture.

MATERIAL AND METHODS

Sections of the portal and splenic veins were examined from 30 cases of cirrhosis of the liver, and from the splenic vein in seven cases in which splenectomy had been performed for recurrent haematemeses or splenomegaly. One of the latter subsequently came to necropsy so that in it the portal vein also was available for study. The microscopic appearance of the portal and splenic veins in two control cases was found to be similar to that noted in the two cases included in the series, in which no pathological changes had occurred and to the description of the normal histology of the veins given by Pei-Lin Li (1940), Maximow and Bloom (1948), Brash and Jamieson (1937), and Brendle (1950).

So far as possible the sections of the portal vein were taken from a point midway between its origin and bifurcation. Sections of the splenic vein were taken from a point midway in its course along the posterior aspect of the pancreas. In the material obtained at operation the only portion of the splenic vein available was the proximal centimetre as it left the spleen.

Transverse sections were stained using, 1, haematoxylin (Meyer's) and eosin; 2, Weigert's elastic tissue stain counterstained with van Gieson's connective tissue stain; 3, Heidenhain's azan.

CHANGES IN PORTAL VEIN

GROUP I: NORMAL PORTAL VEIN Two cases were studied (Fig. 1). The endothelium lining the vein was not always clearly seen but it appeared to be applied directly to the innermost layer of the internal elastic lamina, no subendothelial layer being visible. The elastic tissue of the media ran transversely between the muscle cells in long, thin, wavy strands, the internal elastic lamina being the thickest and best defined. The rest of the media was formed by a compact layer of circular muscle fibres two to four bundles thick and separated from each other by a few loose strands of connective tissue. External to the media and separated from it by a few more connective tissue cells was a loosely formed adventitia composed of two to three small bundles of longitudinally-arranged muscle cells. Between these muscle bundles, running mainly in a longitudinal direction, there was loose connective tissue and a few scattered elastic tissue fibres. External to this again was more loose connective tissue which contained nerve bundles and small blood vessels.

[1]Part of a thesis accepted for the degree of Doctor of Medicine by the University of Edinburgh.

GROUP II: SLIGHT HYPERTROPHY OF MUSCULAR AND ELASTIC TISSUE COATS Eleven cases were studied. There was an increase in the thickness of the vein wall because of slight hypertrophy of all its coats. This increase in thickness is well brought out by comparison of Fig. 1 with Fig. 2. Portions of the vein wall showed no intimal thickening but in other parts there was slight patchy intimal thickening due to the development of small muscle cells in the subendothelial layer. These cells were cut transversely and occurred in small groups with a few thin scattered strands of elastic tissue between the cells. The internal elastic lamina was slightly thickened and stood out more clearly than in the normal vein, while the elastic tissue fibres in the media were also increased in size though not apparently in number. Both the media and adventitial muscle cells were slightly hypertrophied.

GROUP III: MORE MARKED HYPERTROPHY OF MUSCULAR COATS In this group of 15 cases there was definite thickening of the vein wall due to changes in all coats. The intima was diffusely increased in depth and through it were scattered small muscle cells, usually cut transversely, around which ran many thin fibres of elastic tissue, mainly in a circular direction. Through the intima ran many small strands of collagen. The superficial portion of the intima was a homogeneous pale colour and in the lumen of the vein small numbers of red corpuscles were observed adherent to the vein wall, sometimes with a covering of endothelium over them.

The internal elastic lamina was prominent and throughout the media were very many short, thick elastic tissue fibres, cut both longitudinally and transversely. The medial muscles, as well as showing slight hypertrophy, were increased in number so that the media was now six to eight muscle bundles thick.

The adventitial muscles were also slightly hypertrophied, the muscle bundles being larger and more closely arranged than in the normal vessel. Though there was no increase in the number of muscle bundles in the adventitia, the muscle cells were increased both in number and in size. There was no obvious increase in the amount of elastic tissue in the adventitia.

GROUP IV: WELL-MARKED INTIMAL THROMBOSIS IMPOSED ON CHANGES NOTED IN GROUP III The most prominent feature in these three cases was the presence of a large, recently formed thrombus (Fig. 4) on an already eccentrically thickened intima, eccentrically thickened presumably because of previous thrombotic episodes, for it was composed of dense collagenous tissues and the recently formed more superficial thrombus was undergoing organiza-

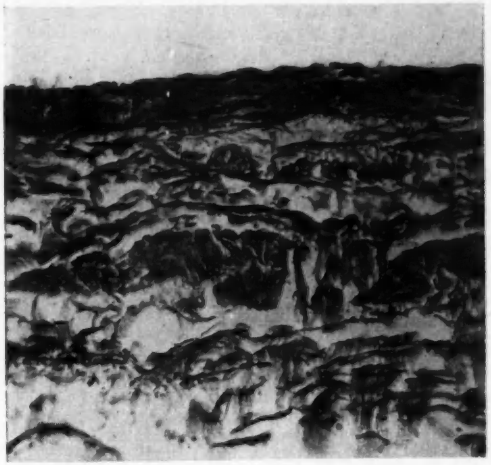

FIG. 1. *A normal portal vein.* (*Weigert and van Gieson.* × *220.*)

FIG. 2. *Portal vein, group II, showing slight hypertrophy of the muscular and elastic tissue coats with minimal intimal changes.* (*Weigert and van Gieson.* × *220.*)

tion by active looking fibroblasts. In the deeper portion of the thickened intima there were many well-formed vascular spaces, and internal to this again many small elastic tissue fibres had proliferated from the neighbouring internal elastic lamina.

The internal elastic lamina was not easily distinguished, for the media was largely replaced by a dense aggregation of elastic tissue amongst which only a few muscle cells remained. External to the media the adventitia consisted of markedly hypertrophied bundles of longitudinal muscle cells cut slightly obliquely.

There was considerable variation in the cases which fell into group IV.

Case 1 There was extensive intimal thickening with recent thrombosis. The adventitia was poorly defined and could scarcely be distinguished, while the most notable feature of the section was the well-marked vascularity of the depths of the intima which contained numerous endothelial-lined vascular spaces and in one part a small arteriole (Fig. 5).

Case 2 The reduplication and fragmentation of the elastic tissue into the deepest portion of the intima was very marked, though the proliferation of elastic tissue in the media was not such a feature as in the two previous cases. In the intima there were one or two small areas of degeneration into which haemorrhage had occurred. The medial and adventitial muscles and the medial elastic tissues were hypertrophied throughout the whole circumference of the vessel.

SPLENIC VEIN

The changes seen at necropsy were of a similar nature but of a slightly less degree than those seen in the portal vein of the same case.

The change seen in biopsies of these veins showed certain characteristics. The adventitial muscle was poorly formed and showed little hypertrophy, while the changes in the medial muscle were more definite but still slight. Intimal changes were, however, present in most cases and there was intimal thickening due to hypertrophy of the same elements as made up the increase in depth in the portal veins and the proximal end of the splenic vein. In two cases the thickening was sharply localized to form raised plaques which projected into the lumen of the vessel (Fig. 6). The changes were similar to those noted and illustrated as occurring in this portion of the vein by McMichael (1935).

RELATIONSHIP BETWEEN CHANGES IN PORTAL AND SPLENIC VEINS AND SIZE OF THE SPLEEN

After the portal and splenic veins had been examined and classified, the changes observed in them were correlated with the size of the spleen (Table I).

TABLE I

Weight of Spleen (g.)	*Changes in the Portal Vein*			
	Group I	*Group II*	*Group III*	*Group IV*
0—199	2	3	1	—
200—399	—	6	7	1
400—599	—	1	7	—
600—799	—	—	—	1
800	—	1	—	1
Totals	2	11	15	3

Finally the pathological changes in the portal veins were correlated with the presence of oesophageal veins and a clinical history of haematemesis (Table II).

DISCUSSION

The association of cirrhosis of the liver, phlebosclerosis of the portal and splenic veins, and splenomegaly was noted by Banti in 1898. He thought that the primary pathological process was in the spleen and that a toxic factor spread thence along the splenic and portal veins to the liver. However, the changes noted by Banti in the spleen have not been described without liver disease (McMichael, 1934) or obstruction in the portal or splenic veins (Kelsey, Robertson, and Giffin, 1947). McMichael described the changes in the veins as hypertrophy of the muscle coats with intimal fibrosis. Subsequently Pei-Lin Li (1940) compared and contrasted the changes in the portal and splenic veins in cases of cirrhosis of the liver with those found in the inferior vena cava under conditions of increased intravenous pressure. He noted that hypertrophy of the medial muscle of the portal vein was often succeeded by intimal changes with the development of small muscle bundles arranged longitudinally in the subendothelial layer, and considered that it was replacement of these muscles by fibrous tissue which produced the thickened and fibrosed intima in some cases. He could find no evidence of any degenerative or

TABLE II

Case no.	*1*	*2*	*3*	*5*	*8*	*13*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*23*	*24*	*25*	*26*	*31*
Varicose veins	+	+	+	−	−	+	−	+	−	+	+	+	−	−	+	−	+	+
Haematemesis	−	+	−	−	−	+	−	+	−	−	+	−	−	−	+	−	−	−
Vein changes	IV	IV	III	III	III	III	III	III	III	III	III	III	III	III	IV	III	III	III

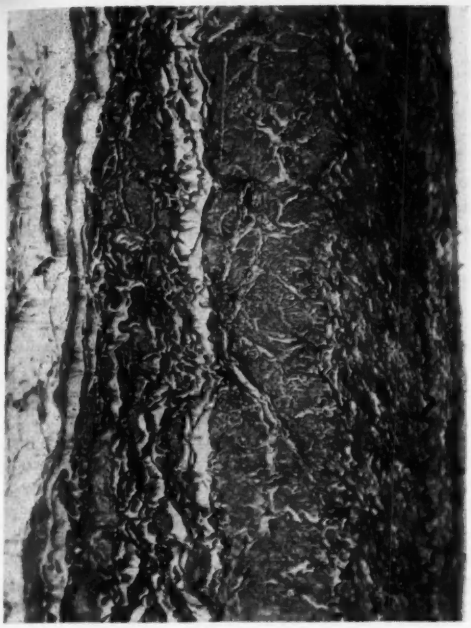

FIG. 3

FIG. 4

FIG. 3. *Portal vein, group III, with more marked hypertrophy of the muscular coats and definite, usually diffuse, intimal thickening. (Weigert and van Gieson. × 80.)*

FIG. 4. *Portal vein, group IV, showing well-marked intimal thrombosis superimposed on the changes noted in group III. (Weigert and van Gieson. × 15.)*

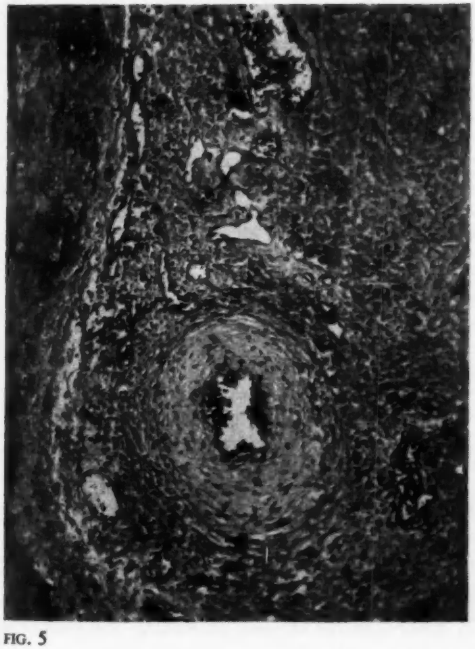

FIG. 5

FIG. 6

FIG. 5. *Vascularization of the deep portions of the thrombus adjacent to the internal elastic lamina. (Haematoxylin and eosin × 80.)*

FIG. 6. *Localized thickening of the splenic vein (Azan. × 80.)*

atheromatous change. Since veins were found with hypertrophy of the medial muscle and no intimal thickening but never intimal hypertrophy without medial muscle hypertrophy, it was assumed that the change in the medial muscle preceded that in the intima. The changes too in the splenic vein were always of a slighter degree than those in the portal vein of the same case and comparison of the lesions in these two vessels afforded further support for his contention.

These findings have been confirmed. In contradistinction, however, to Pei-Lin Li's conclusion that where there is an increased venous pressure the hypertrophy of the adventitial muscle could not be regarded as significant, the degrees of hypertrophy in the medial and adventitial muscle coats were always noted to be equal. Accurate measurement of the adventitia was difficult, for its boundaries were often ill defined and the whole loosely arranged. However, since the hypertrophy of the muscle bundles occurred at the expense of the loose connective tissues rather than by any increase in width of the coat as a whole (Figs. 1, 2, and 3), the measurement of the width of the coat as carried out by Pei-Lin Li was not considered a satisfactory index of its muscular hypertrophy.

Furthermore, the changes in the elastic tissue of the media were noted to be of roughly the same extent as the accompanying hypertrophy of the medial muscles, this increase in the amount of elastic tissue and an increasingly well-defined internal lamina being often the first and most easily observed change in these veins (Fig. 2). Where the elastic tissue changes were most marked, amounting to an elastosis of the media (Fig. 4), the factor responsible for these lesions had probably produced its greatest effect. The most marked elastic tissue changes in the media were noted where there was extensive intimal thrombosis, suggesting that the thrombosis too was a result of this same factor, probably prolonged and excessive pressure in the vein. However, the occurrence of thrombosis in a vein depends on so many factors (Rosenthal, 1925; Evans, 1929; Simonds, 1936; Reich, 1942), and the fact that the muscular hypertrophy was sometimes most marked below the greatest intimal thickening, combine to make it difficult to decide the relative importance of these findings. In spite of this, and since in the cases with intimal thrombosis the muscle coats and elastic tissue were also markedly hypertrophied, it seems reasonable to suggest that these changes are the result of prolonged congestion. The four groups of venous changes described probably do represent successive stages of a process in the vein wall, the hypertrophy of the internal elastic lamina and the medial muscle being followed by further hypertrophy of both medial and adventitial muscle and elastic tissue with an increase in depth of the subendothelial layer, patchy at first and later becoming more diffuse. A third stage in the process has been reached in those cases in which the hypertrophy of these coats is most marked. Extensive intimal thrombosis is probably a terminal occurrence in this chain of events.

These changes were at one time thought to be due to a weakening of the vein wall by inflammation (Fischer, 1900; Warthin, 1910; Faber, 1912), while Simmonds (1912) distinguished a group in which syphilis was thought to be important. However, no evidence of inflammation was found in any of the cases examined. A further cause of these changes has been suggested as being a lack of nutritional factors with subsequent weakening of the vein wall. However, similar changes are found in veins in which there is a definite increase in pressure, for instance, in arteriovenous aneurysms (Hermannes, 1923), and in 11 of the 31 cases examined a collateral circulation had developed around the oesophagus, a finding which McMichael (1934) considered definite proof of an increased pressure in the portal vein, its presence during life making a clinical diagnosis of portal hypertension possible (Reynell, 1951).

If, as has been suggested, the presence of varicose veins around the oesophagus denotes an increased pressure in the portal vein and the changes noted in the veins examined are due to that increased pressure, then the most marked changes in the veins should be associated with oesophageal varices. Examination of the findings from the material does bear this out (Table II), thus adding further support to the contention that the changes in group IV occur after those in group III.

Some light was also thrown on the aetiology of the intimal thickening. In 10 of the cases examined red blood cells were seen firmly adherent to the vein wall, and in one or two cases endothelium had grown over them. In the three cases belonging to group IV, the thrombus appeared to be superimposed on a previous older lesion, for muscle cells and elastic tissue were not present in the eccentrically thickened portion of the intima. The mechanism whereby these intimal changes arise may be analogous to that described by Duguid (1946) in the formation of atheromatous thickening in the walls of arteries where thrombosis and subsequent overgrowth of endothelium also occurs. Providing additional support for this contention are the facts that where the intimal thickening was still of small amount, the increase in depth was caused by hypertrophy of all elements (group III), but where the thickening was more marked, that is in group IV, the intimal thickening was eccentric

and was formed of fibrous tissue with, in its depths, many small endothelial-lined vascular channels and in one case a small, well-formed arteriole (Fig. 5). This marked vascularity of the depths of the intima probably accounts for the infrequency with which degenerative intimal changes were noted, though in Case 2 small areas of necrosis were seen in the thickened intima and the calcification in two cases was presumably the result of a similar process in a part of the vein where the thrombus was deepest.

If the extent of the splenomegaly associated with cirrhosis of the liver depends only on the extent or duration of the increased portal vein pressure, the changes in the vein wall will show a direct relationship to the size of the spleen. Study of the portal veins examined in this investigation should therefore enable a decision to be reached as to the relative importance of congestion and hyperplasia (Table I).

Table I includes two cases in which the changes were so different as to deserve separate consideration. In the first (male, aged 47) the splenic pulp showed no hyperplasia at all, the spleen weighing 40 g. and being noted as showing atrophy, yet group III changes were present in the portal vein, while in the second (male, aged 75) only slight changes (group II) were present in the portal vein yet there was marked hyperplasia of the splenic pulp (weight 970 g.) without fibrosis or any other evidence of congestion. Probably the factor which caused the liver damage in the latter case, perhaps not long before death, had at the same time affected the spleen (cf. Menon, 1938). However, apart from these two cases there is a definite relationship between the changes in the portal vein and in the spleen. It appears therefore that the extent of the splenomegaly which occurs in cirrhosis of the liver is usually related to the extent of the changes in the portal vein, though other factors play an unknown and presumably variable part: in other words, the same factor which causes the changes in the veins is responsible for part of the splenomegaly. Whether this factor is the degree or duration of the rise in portal pressure is difficult to ascertain, but the size of the spleens and the respective ages of the two cases commented on above suggest that the age is not an important factor in deciding the extent of the splenomegaly.

SUMMARY

The portal and splenic veins in 31 cases of cirrhosis of the liver were examined and the changes occurring in them classified into four main groups. These groups and the different stages of their production were considered to give an indication as to the degree or duration of the congestion, a finding supported by the relationship between these changes and the presence of a collateral circulation.

In the group with the most marked changes in the vein walls, thrombosis was a feature.

Furthermore, there was a relationship between the different changes in the portal and splenic veins and the size of the spleen in portal hypertension. The more marked the changes in the veins, the larger the spleen. The conclusion is therefore reached that the changes in the veins and in the spleen in cases of portal hypertension reflect the degree of duration of that increase in portal pressure.

The material for this investigation was obtained from the Pathology Department of the Royal Infirmary, Edinburgh, and Edinburgh University. I have to thank Professor A. M. Drennan and Dr. R. F. Ogilvie for their kind encouragement and help and also Sir James Learmonth for access to much interesting case material. The photomicrographs are the work of Mr. T. C. Dodds.

REFERENCES

Banti, G. (1898). Splenomegalie mit Lebercirrhose. *Beitr. path. Anat.*, **24**, 21-33.

Brash, J. C., and Jamieson, E. B. (1937). *Cunningham's Text-book of Anatomy*, 7th ed. Oxford University Press, London.

Brendle, E. (1950). Über den Bau der menschlichen Pfortader und ihrer Wurzeln. *Acta anat. (Basel)*, **10**, 108-129.

Duguid, J. B. (1946). Thrombosis as a factor in the pathogenesis of coronary atherosclerosis. *J. Path. Bact.*, **58**, 207-212.

Evans, W. H. (1929). The blood-platelets in splenic anaemia, with special reference to treatment by splenectomy. *Lancet*, **1**, 1, 277-282.

Faber, A. (1912). *Die Arteriosklerose: ihre pathologische Anatomie, ihre Pathogenese und Aetiologie*, p. 122. Fisches, Jena.

Fischer, B. (1900). Ueber Entzündung, Sklerose und Erweiterung der Venen mit besonderer Berücksichtigung des elastischen Gewebes der Gefässwand. *Beitr. path. Anat.*, **27**, 494-554.

Hermannes, P. (1923). Zur Frage der arterialisierten Venen beim arterio-venösen Aneurysma. *Beitr. path Chir.*, **130**, 40-59.

Kelsey, M. P., Robertson, H. E., and Giffin, H. Z. (1947). The rôle of chronic thrombosis of the portal vein and its tributaries in the syndrome of splenic anaemia. *Surg. Gynec. Obstet.*, **85**, 289-293.

Li, Pei-Lin (1940). Adaptation in veins to increased intravenous pressure, with special reference to the portal system and inferior vena cava. *J. Path. Bact.*, **50**, 121-136.

McMichael, J. (1934). The pathology of hepatolienal fibrosis. *Ibid.*, **39**, 481-502.

——. (1935). Splenic anaemia. *Trans. med.-chir. Soc. Edinb.*, 1934-35, 97-116.

Maximow, A. A., and Bloom, W. (1948). *Textbook of Histology*, 5th ed. W. B. Saunders, Philadelphia and London.

Menon, T. B. (1938). The splenic reaction in experimental cirrhosis and in pre-cirrhotic intoxication. *J. Path. Bact.*, **46**, 521-534.

Reich, N. E. (1942). Portal system thrombosis occurring in portal hypertension. *Ann. intern. Med.*, **17**, 270-294.

Reynell, P. C. (1951). The prognosis of portal hypertension. *Lancet*, **2**, 383-385.

Rosenthal, N. (1925). Clinical and hematologic studies on Banti's disease. 1. The blood platelet factor with reference to splenectomy. *J. Amer. med. Ass.*, **84**, 1887-1891.

Simonds, J. P. (1936). Chronic occlusion of the portal vein. *Arch. Surg. (Chicago)*, **33**, 397-424.

Simmonds, M. (1912). Über Pfortaderskerose. *Virchows Arch. path. Anat.*, **207**, 360-373.

Warthin, A. S. (1910). The relation of thrombophlebitis of the portal and splenic veins to splenic anaemia and Banti's disease *Int. Clin.*, 20th ser., **4**, 189-226.

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Radiological investigation of the small intestine

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SYNOPSIS This paper reports experience with a selective technique of small intestine enemas.

In the past, radiological investigation of the small intestine has relied almost exclusively upon the barium follow-through series. Many variations have been devised to ensure a complete and even filling but none has advanced the accuracy of radiological diagnosis. This technique has failed too often to warrant complacency and an analysis of the causes of failure may help to encourage the adoption of an alternative method of examination.

Certain disadvantages are obvious. The filling of the small intestine is controlled by the rate of delivery through the pylorus and this is unpredictable within each interval of the serial examination. It tends to produce irregular filling and any irritability of the bowel itself further complicates the picture by producing fragmentation and flocculation of the barium column. The opaque coils, by overlapping, may obscure a small lesion. Each film of the series is only a demonstration of the appearances in a moment of time and the pathological segment may never be seen.

These factors militate against a complete survey of the tract but we consider the real limitation of this form of examination to be due to inability to test elasticity of the bowel wall. The barium column in the follow-through series is propelled forward by peristaltic activity and at the same time the maximum diameter of the lumen is controlled by the tone prevailing at the moment. Even following partial gastrectomy, where pyloric control is removed, the intestinal passage of barium is still under the influence of peristaltic activity and the tone remains dominant. Obviously an early lesion, causing minimal constriction, will not be visible if the constriction has not yet reduced the lumen below that permitted by the tone prevailing. A constrictive lesion developing very slowly causes hypertrophy of the proximal bowel wall and this overcomes the tendency to dilate against the obstruction.

Badenoch (1960) stressed the importance of careful radiological examination in every patient suffering from steatorrhoea in the search for intrinsic pathology of the small bowel. He stated, however, that some structural lesions are extremely difficult to demonstrate. Blind loops may be overlooked and fistulae are often difficult to find; diverticula also are frequently unrecognized. Even if some diverticula do fill it is rare to have a complete picture of the total extent of a generalized diverticulosis by means of the follow-through series. We think that this failure to demonstrate diverticula and fistulae is due also to the maintenance of tone and peristaltic activity which prevents the entry of barium into them during the passage of the bolus.

The definition of adhesions may also cause difficulty. Here the diagnosis depends upon the ability to test mobility in each segment of the small bowel in progression, with the patient in varying degrees of posture from the horizontal to the vertical, in order to demonstrate the persistence of an acute kink under conditions of rapid intestinal flow.

Finally, problems of radiological diagnosis arise in the proximal colon when filling by the retrograde colon enema is found to be impossible. In these cases a true definition of the extent and the appearances of the pathological segment is not obtained.

If this is a correct interpretation of the limitations of the ingested opaque meal, relying on peristalsis for progression, the success must depend upon a technique which will be independent of peristaltic activity and prevailing tone. At the same time, the disadvantage of obscuring bowel segments by overlapping must be avoided and, if possible, a double contrast technique must be sought to give a clear picture of the mucosal pattern.

Schatzki (1943) devised an alternative technique which he called the small bowel enema. It was designed to overcome the disadvantage of pyloric control by the introduction of a continuous flow of opaque medium beyond the pylorus, but this method did not gain favour on two counts: 1 The difficulty of attaining duodenal intubation, which occupied trained staff for a protracted period

without adequate certainty of success; and 2 the disadvantage already mentioned in relation to the follow-through series, namely opacification of the whole small bowel. Greenspon and Lentino (1960) have devised a method of retrograde enterography using a modified Miller-Abbott tube, but this requires the passage of the tube far down the small bowel which adds to the complexity of the procedure.

We report here a variety of lesions of the small bowel demonstrated since the introduction of a simple small bowel enema technique as a standard procedure in this department six years ago. The report includes cases of inflammatory disease, diverticulosis and adhesions of the small bowel, and a case of carcinoma of the ascending colon. No case of tumour of the small bowel has been referred for examination within the period of this series.

The technique of duodenal intubation and the method of achieving a double-contrast small bowel enema are described in detail elsewhere (Scott-Harden, 1960). We have limited the quantity of opaque medium to the equivalent of 20 ml. of Microtrast diluted to 80 ml. with tap water. This amount is only increased when the review of a questionable segment is necessary. A greater quantity tends to cause errors of interpretation by overlay of the opaque coils. Onward progress of the opaque column is achieved by the subsequent injection of tap water and the barium left behind adhering to the mucosa serves to give double contrast.

Modifications of the design of the tube and the enema technique have been reported by Pygott, Street, Shellshear, and Rhodes (1960). One can hope that experiment will evolve further improvements. It is our practice to undertake this procedure, requiring no more than 45 minutes, in the out-patient department and experience has shown no disadvantage.

CASE REPORTS

CASE 1 R.K., a man aged 45 years, referred by Dr. T. McL. Galloway, was first seen early in 1955 complaining of vague dyspepsia. A barium meal and follow-through at that time failed to demonstrate any ulceration in the stomach or duodenum or demonstrable abnormality of the small intestine. Persistent symptoms and the onset of intestinal haemorrhage called for review in October of the same year, when a small bowel enema showed a narrow segment of jejunum with localized ulceration (Fig. 1). Operation by Mr. J. E. Monro confirmed the lesion which was resected and continuity re-established by end-to-end anastomosis. No other lesion of the small intestine was found at this operation and the specimen was reported by Dr. J. S. Faulds to be of Crohn's disease.

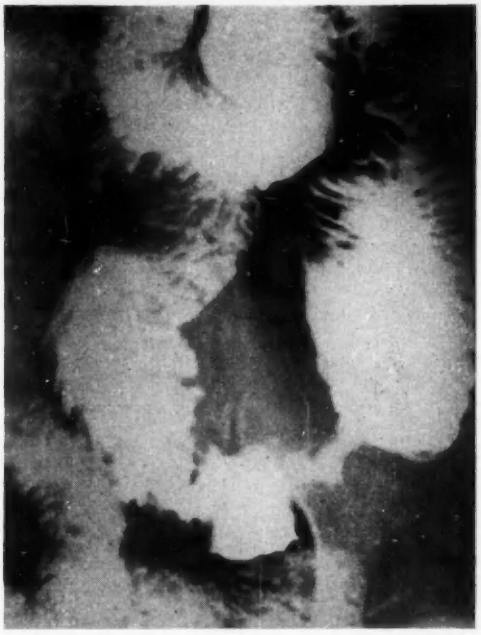

FIG. 1. *Case 1: Small bowel enema showing rigid narrowed segment of jejunum contrasted by complete filling of the bowel on each side of the constriction. Crohn's disease.*

CASE 2 T.H., a man aged 42, was referred by Dr. Hugh Morton in 1956. This case serves to contrast the appearances of a pathological segment of jejunum in the follow-through series and the double-contrast small bowel enema. In the follow-through series a long segment of jejunum appears as a rigid tube with a cobblestone mucosal pattern (Fig. 2). The small bowel enema, however, shows that this segment retains some degree of elasticity and the cobblestone pattern of the mucosa is ironed out as the dilatation occurs, only to return with the natural contraction when the injection is discontinued (Figs. 3a and 3b). This suggested that the cobblestone pattern was due to the inability of the mucosal lining to crumple up on account of submucosal oedema or infiltration and was interpreted as non-sclerosing enteritis. No surgical interference was considered and the patient continues at work to the present time.

CASE 3 R.E., a woman aged 49, was referred by Mr. R. M. Hill in 1959. Nine years previously a hemicolectomy had been performed for Crohn's disease. She remained well until March 1959 when she complained of pain in the right iliac fossa and was found to have a tender mass in that area. The retrograde colon enema

FIG. 2. *Case 2: Barium meal and follow-through showing segment of jejunum with cobblestone mucosa and appearing rigid. Non-sclerosing enteritis.*

became totally obstructed in the proximal transverse area and no further information was forthcoming. A small bowel enema showed a rigid diseased segment of ileum and an extraluminal track running into the area of the mass. Also a loop of proximal ileum was seen adhering to the mass (Figs. 4a and 4b). Mr. Hill resected the diseased ileum and colon and the patient's subsequent progress has been satisfactory without suspicion of recurrence so far. The specimen was reported to be of Crohn's disease.

CASE 4 A.M., a woman aged 62 years, was referred by Mr. J. E. Monro in 1960 for investigation of suspected subacute intestinal obstruction. The colon enema failed to penetrate beyond the proximal transverse segment and did not define the nature of the lesion. A small bowel enema six days later showed conclusive evidence of a carcinoma of the ascending colon producing the typical bracket deformity (Fig. 5). This lesion was found to be inoperable and a short circuit only was possible.

CASE 5 T.H., a man aged 41 years, was referred by Mr. A. B. MacLean with a history of episodic upper abdominal pain for five years with occasional vomiting. The bowels were generally regular and the stools normal in consistence and colour except for occasional bursts of diarrhoea. The clinical examination was essentially negative.

The barium meal and follow-through showed no evidence of peptic ulceration in the stomach or duodenum but demonstrated five or six diverticula scattered in the jejunum. A small bowel enema showed very numerous diverticula throughout the whole length of the small

FIG. 3a. *Case 2: Small bowel enema with double contrast showing some elasticity and the mucosa no longer cobblestoned.*

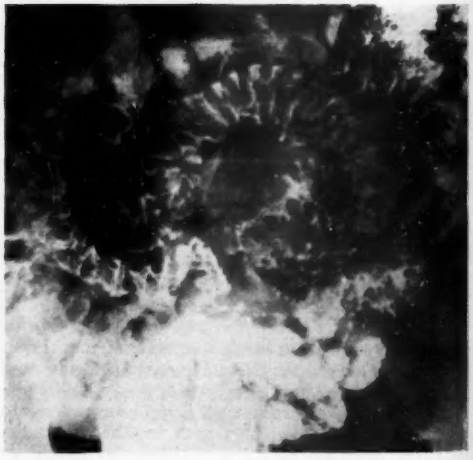

FIG. 3b. *Case 2: Same segment of jejunum as in Fig. 3a, showing return of cobblestone pattern as contraction takes place when injection is discontinued.*

FIG. 4a

FIG. 4b

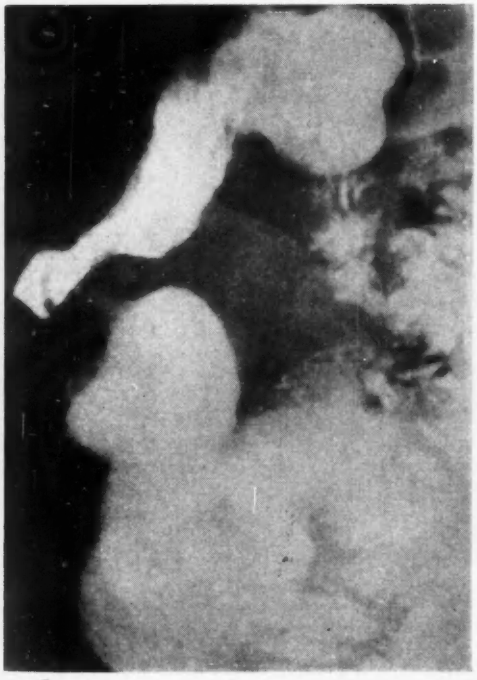

FIG. 5

FIG. 4a. *Case 3: Small bowel enema showing filling of terimal ileum with rigid, narrowed segment. Crohn's disease. Note also adhesion of a proximal coil giving the typical acute kink and mucosal pattern.*

FIG. 4b. *Case 3: A later stage of injection than in Fig. 4a showing filling of the colon and extraluminal track. Note adhesion again defined.*

FIG. 5. *Case 4: Small bowel enema showing typical deformity of carcinoma of colon.*

FIG. 6a. *Case 5: Small bowel enema showing multiple jejunal diverticula. Note double contrast filling in the upper group.*

FIG. 6b. *Case 5: Small bowel enema showing multiple diverticula in the lower jejunum and ileum. Note thin barium opacity allowing definition in spite of overlap.*

FIG. 7a

FIG. 7b

FIG. 7c

FIGS. 7a, 7b, and 7c. Radiographs of *Case 6.*

7a. *Small bowel enema demonstrating jejunal adhesion and showing loop of jejunum with smooth curve when the patient was supine.*

7b. *Slight tenting of loop with 45° table tilt.*

7c. *Acute kink of loop with patient erect.*

FIG. 8. *Case 7: Small bowel enema showing jejunal adhesion as acute kink of loop and mucosal contrast pattern typical of adhesions tenting in the erect posture.*

intestine in numbers quite unsuspected in the follow-through series (Figs. 6a and 6b).

CASE 6 W.M., a man aged 42 years, was referred by Dr. J. Simpson in February 1961 giving a history of gastrectomy for chronic duodenal ulceration eight years previously. Ever since a stormy post-operative recovery he has complained of vomiting bile and of a consciousness of abdominal distension after meals. A small bowel enema demonstrated an adhesion of the jejunum which was not recognized in the supine posture but became increasingly obvious as the table tilt moved to the vertical (Figs. 7a, 7b, and 7c). Operation by Mr. A. M. Loughran confirmed this adhesion which was separated.

CASE 7 T.K., a man aged 57 years, was referred by Mr. G. H. D. McNaught who had undertaken an emergency gastrectomy in February 1960. Immediate post-operative recovery was uneventful. The patient remained well until June when he developed abdominal pain reaching a climax in four hours necessitating admission to hospital as an emergency. A Gastrografin meal on admission showed no gastrojejunal ulceration. The acute phase settled with conservative treatment and the patient was referred for a small bowel enema which revealed a single acute kink in a loop of jejunum, accentuated in the erect posture, consistent with an adhesion causing sling fixation (Fig. 8). Operation by Mr. McNaught confirmed the presence of an adhesion which was a thick fleshy band attaching the loop of jejunum to the undersurface of the liver.

CASE 8 J.A., a man aged 38, was referred by Mr. J. E. Monro and admitted to hospital with a picture of

FIGS. 9a and 9b. *Case 8: Small bowel enema showing two stages of filling to reveal partial ileal obstruction.*

subacute obstruction and a history of a gunshot wound of the abdomen in 1944. The obstructive symptons were relieved by conservative treatment and the patient was referred for a small bowel enema which showed partial obstruction in the ileum where the coils were matted together. The total length of the small intestine appeared abnormal suggesting that there had been a considerable resection and this was later confirmed from Army records. Operation was considered undesirable, if it could be avoided, and the patient has been kept under careful review and serious symptoms have not recurred to date (Figs. 9a and 9b).

CONCLUSION

We consider that the follow-through series can only be accepted should a definite positive finding result. A negative follow-through series should not be allowed to lead to a sense of security, so often proved to be false. We recommend the small bowel enema in any case in which the clinician suspects pathology of the intestinal tract and in which the barium meal and colon enema proved negative. It is not, in fact, and should not be, considered any more specialized a procedure than the double-contrast colon enema and we have not found it more time consuming. Certainly it cannot be excluded from the diagnostic armament in any case of obscure occult bleeding. Experience has shown that advantage lies in using a minimum of barium in order to prevent any possibility of overshadowing a small diseased segment. Finally, we would stress the necessity to examine with varying degrees of table tilt to ensure that an acute kink, due to an adhesion, is demonstrated. It is also of interest that the removal of the pylorus by gastrectomy does not allow satisfactory investigation of the small bowel by the ingested meal and intubation of the efferent loop is still necessary.

We wish to express our thanks to our clinical colleagues mentioned in the text and to Drs. Studdert and Rolland and Mr. Jamieson who also referred cases in their clinical charge for investigation. This method of examination could not have been carried forward without their encouragement. We would also thank Haley's of Carlisle for the great care taken in the preparation of the reproductions. Finally, we would record our appreciation to the radiographic staff of the Cumberland Infirmary for their help throughout this investigation.

REFERENCES

Badenoch, J. (1960). Steatorrhoea in the adult. *Brit. med. J.*, **2**, 963-974.

Greenspon, E. A., and Lentino, W. (1960). Retrograde enterography. A new method of roentgenologic study of the small bowel. *Amer. J. Roentgenol.*, **83**, 909-918.

Pygott, F., Street, D. F., Shellshear, M. F., and Rhodes, C. J. (1960). Radiological investigation of the small intestine by small bowel enema technique. *Gut*, **1**, 366-370.

Schatzki, R. (1943). *Amer. J. Roentgenol.*, **50**, 743.

Scott-Harden, W. G. (1960). In *Modern Trends in Diagnostic Radiology (Third Series)*. Ed. J. W. McLaren, p. 84. Butterworth, London.

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The intestinal absorption defect in cystinuria

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SYNOPSIS This paper brings forward evidence to suggest that the amino-acid transport defect known to occur in the kidneys of cystinurics is also present in the gut.

Cystinuria was first described 150 years ago (Wollaston, 1810) and numerous theories have since been advanced to explain the clinical and biochemical abnormalities of the disease. The present view of the disorder (Dent and Rose, 1951) is that cystinuria is a disease of the kidney tubules, involving reduced reabsorption by the renal tubule cells from the glomerular filtrate of the dibasic amino-acids—cystine, lysine, arginine, and ornithine. No other tubular defect has been found, implying a specific defect of amino-acid transport in kidney cells. We consider this to be a correct but incomplete view of the disordered physiology of the disease, and describe experimental evidence for a more generalized defect, possibly involving amino-acid transport in all body cells. This wider concept explains many previous observations not easily reconciled with the current more restricted view of the disease.

Cystinuria is a recessive hereditary disease (Dent and Harris, 1951; Harris and Warren, 1953; Harris, Mittwoch, Robson, and Warren, 1955a and b; Harris and Robson, 1955), such conditions usually involving a deficiency or absence of a single protein, which may be an enzyme. The biochemical defect in Hartnup disease (Baron, Dent, Harris, Hart, and Jepson, 1956; Milne, Crawford, Girão, and Loughridge, 1960), a comparable recessive hereditary condition, is a reduced ability to transport those amino-acids which are not involved in the transport defect of cystinuria. The defect in Hartnup disease has been shown to involve cells both of the proximal renal tubules and of the jejunum. This paper presents evidence that cystinuria and Hartnup disease are closely related conditions, each comprising a specific hereditary disorder of amino-acid transport. The dibasic amino-acids—cystine, lysine, arginine, and ornithine—are involved in cystinuria, and most of the other amino-acids derived from protein hydrolysis, with the notable exceptions of glycine and proline, in Hartnup disease.

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MINOR METABOLIC PATHWAYS OF LYSINE AND ORNITHINE

Our experimental plan makes use of minor pathways of lysine and ornithine metabolism and is impossible to explain without a previous short review of existing knowledge. Lysine, if not utilized in protein synthesis, is normally oxidized to α-keto-ε-amino-caproic acid. This is then metabolized by a chain of reactions to α-ketoglutaric acid, and thus to the tricarboxylic acid cycle (Rothstein and Miller, 1954). Arginine and ornithine are involved in the cyclic series of reactions in the formation of urea (Krebs and Henseleit, 1932). The present work is not concerned with these major pathways of metabolism.

Many bacteria, including numerous organisms of the normal colonic flora, break down lysine, arginine, and ornithine, by the action of specific decarboxylases, to the corresponding diamines—cadaverine, agmatine, and putrescine. These decarboxylases are most active at an acid reaction (Gale, 1940a), the optimum *p*H being 4·0 for arginine decarboxylase, 4·5 for lysine decarboxylase, and 5·0 for ornithine decarboxylase. In this investigation we have never detected agmatine either in faeces or urine, either because arginine itself was not administered or because of the low *p*H optimum of the appropriate decarboxylase. The formation and further metabolism of cadaverine and putrescine is shown to be of considerable importance in cystinuria, confirming earlier work (von Udránszky and Baumann, 1889; Garrod, 1908). A summary scheme of the further metabolism of the diamines is given in Fig. 1.

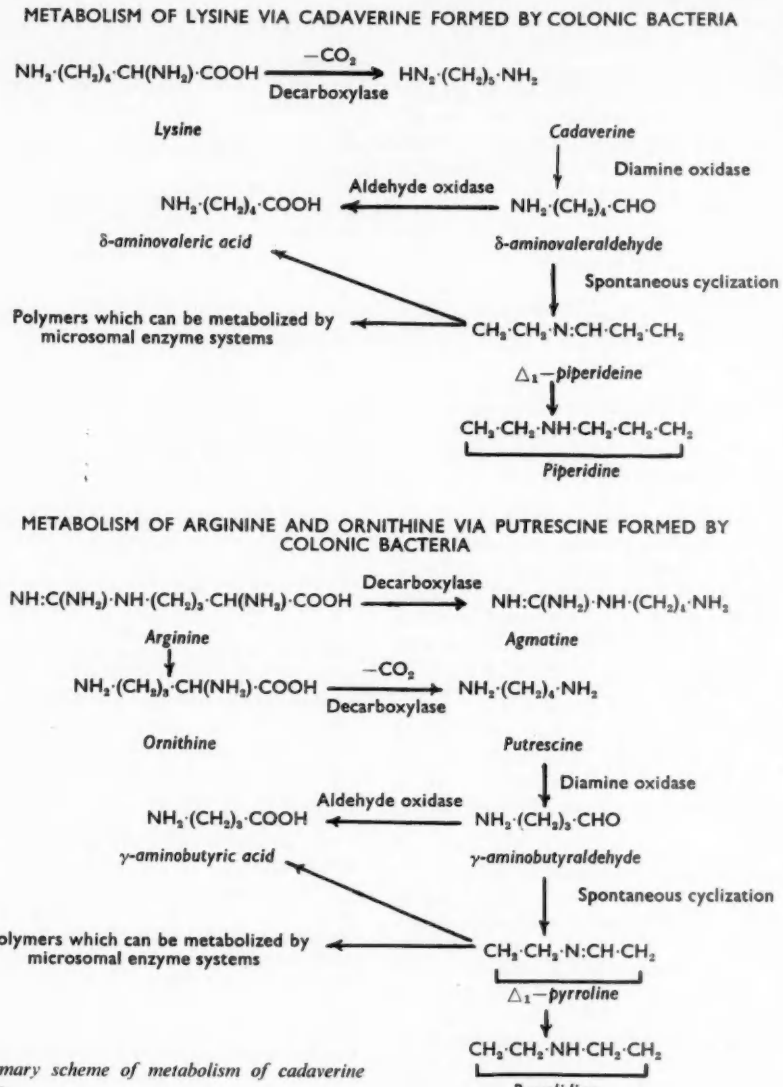

FIG. 1. *Summary scheme of metabolism of cadaverine and putrescine.*

The diamines are readily absorbed from the colon, and are oxidized by diamine oxidase to the corresponding aldehydes, δ-aminovaleraldehyde and γ-aminobutyraldehyde. The greater proportion of these aldehydes is oxidized by aldehyde oxidase to the corresponding amino-acids, δ-aminovaleric acid and γ-aminobutyric acid, both of which can be fully metabolized to CO_2 and urea. The aldehydes, however, are highly reactive compounds, and spontaneously cyclize to the unsaturated heterocyclic amines, Δ_1-piperideine and Δ_1-pyrroline. Polymers of these amines are formed when the diamines are incubated with diamine oxidase of plant origin (Mann and Smithies, 1955). Suzuki and Hagihara

(1954) performed similar experiments using diamine oxidase prepared from hog kidney, and claimed that the saturated heterocyclic amines, piperidine and pyrrolidine, were formed. Asatoor and Milne (1961) confirmed the production of these saturated amines, but also detected considerable quantities of the polymers of Δ_1-piperideine and Δ_1-pyrroline by use of the methods of Mann and Smithies (1955). When injected into rats these polymers were found to be rapidly metabolized by microsomal enzyme systems, only 1-2% of the injected dose being excreted in the urine. Strecker (1960) has recently shown that both Δ_1-piperideine and Δ_1-pyrroline can also be oxidized by a mitochondrial enzyme to δ-aminovaleric and γ-aminobutyric acids respectively. The highly reactive unsaturated heterocyclic amines are therefore probably metabolized in three ways: 1, Spontaneous polymerization, and metabolism of the polymers by microsomal enzymes; 2, oxidation by a mitochondrial enzyme to ω-aminoacids; and 3, reduction to the saturated heterocyclic amines, piperidine and pyrrolidine.

The urinary excretion of these saturated heterocyclic amines by man was first noted by von Euler (1945), who recorded a total output of 3 to 20 mg. per day. This paper shows that formation of piperidine is dependent on bacterial metabolism within the colon of unabsorbed lysine to cadaverine, and that pyrrolidine formation depends on similar bacterial metabolism of arginine and ornithine. The reaction chains are completely specific, and therefore pyrrolidine cannot be formed from lysine nor can piperidine from arginine or ornithine. Colonic bacteria can readily convert arginine to ornithine (Gale, 1940b) and, probably because of the highly acidic *p*H optimum of arginine decarboxylase, this is a more important metabolic pathway that direct decarboxylation of arginine to agmatine. Excess excretion of the saturated heterocyclic amines by cystinurics is shown to be an important but hitherto undescribed aspect of the disease.

METHODS

Metabolic tests were carried out in five homozygous cystinuric patients, all giving a history of urinary calculus disease. One was a child of 8 and the others were adults. The results were compared with similar tests in 12 normal adult control subjects without evidence of renal or metabolic disease. Each test consisted of oral ingestion of a solution of either L-lysine or L-ornithine monohydrochloride, followed by serial analyses of urine and faecal specimens passed during the next 24 hours. Preliminary tests using 4 g. L-lysine hydrochloride gave equivocal results and are not reported. L-lysine, 20 g., was then given as two doses of 10 g. at an interval of four hours. Half the dose was used in the cystinuric child. One cystinuric patient was given the same dose of L-ornithine hydrochloride, but this caused diarrhoea and abdominal colic. As reported later, the amount of potentially toxic metabolites produced was much greater than after an identical dose of L-lysine hydrochloride. In the other tests using L-ornithine hydrochloride, a single dose of 10 g. was used.

The 24-hour metabolic tests to be reported comprise (*a*) tests on four cystinuric patients and 10 normal subjects receiving L-lysine; (*b*) one cystinuric patient and one normal subject receiving L-lysine after pretreatment for three days with oral neomycin, 10 g. daily; (*c*) two cystinuric patients receiving L-ornithine, 20 g. and 10 g. respectively, and two normal subjects receiving the smaller dose.

A two-hour basal specimen of urine was collected, in most cases from 7 a.m. to 9 a.m. The first dose of the amino-acid was then given, and separate two-hourly urine specimens were collected for the next 12 hours. Finally, an overnight 12-hour urine specimen was obtained. Separate faecal specimens were obtained, including the last specimen passed before the test, and all specimens passed spontaneously during the 24 hours of the metabolic tests. If faeces were not obtained during the 24-hour period, a glycerine suppository was given at the end of the test. All urine and faecal specimens were deep frozen immediately after collection.

CHEMICAL METHODS

AMINO-ACIDS IN FAECES The faeces were homogenized with four times their weight of acetone and then filtered. The acetone was evaporated off under reduced pressure, and the residue diluted with distilled water to a volume in millilitres equivalent to half the original faecal weight in grams. The resultant extract was filtered and 25 μl. chromatographed using butanol:acetic acid:water, 4:1:2, as solvent. The amino-acid spots were developed either by ninhydrin or by a diacetyl-naphthol reagent for the guanidino-group.

DIAMINES IN FAECES The remainder of the faecal extract was mixed with one-fifth its volume of saturated ammonium reineckate solution. After standing for two hours the precipitated amine reineckates were removed by centrifugation and purified by recrystallization from acetone. A solution of the purified reineckates in acetone was then chromatographed using the same technique as described for faecal amino-acids.

DINITROPHENYL DERIVATIVES OF URINARY AMINES Dinitrophenyl (DNP) derivatives of urinary amines were prepared as described by Asatoor and Kerr (1961) and their subsequent chromatography as described by Asatoor (1960).

QUANTITATIVE ASSESSMENT OF TOTAL HETEROCYCLIC AMINE CONTENT OF URINE The amount of urine corresponding to a 10-minute period was diluted to 25 ml. with distilled water. This was saturated with excess NaCl and 5 ml. 20% NaOH was added. The

solution was extracted with 20 ml. chloroform. The chloroform layer was separated by centrifugation and dried over anhydrous sodium sulphate. The solution was then shaken with an equal volume of N/10 HCl. Two ml. of the acid phase was then neutralized with N NaOH and the solution made up to 3 ml. with distilled water. To this was added 2 ml. 2% sodium borate, 5 ml. ethanol, and 1 ml. freshly prepared 0·5% solution of the sodium salt of 1:2 naphthoquinone-4-sulphonic acid. After development at room temperature for 15 minutes, the colours were compared with standard piperidine or pyrrolidine solutions treated in an identical manner in a Unicam spectrophotometer at wavelengths 500 and 560 mμ. The absorption spectrum of the colour obtained from the heterocyclic amines has a steep gradient between these wavelengths, and maximum specificity was obtained by using the difference between the two readings. Piperidine standards were used in the experiments involving lysine, and pyrrolidine standards after ornithine. There was only a slight difference in the absorption obtained from each heterocyclic amine per mole. Cadaverine, but not putrescine or agmatine, was found to interfere somewhat in the method. An olive green colour is obtained from cadaverine in contrast to the rose colour produced by the heterocyclic amines. By comparing the absorption spectra of the solutions it was always possible to recognize urine specimens containing cadaverine as well as the more ubiquitous heterocyclic amines. The sensitivity of the method for cadaverine per mole was one-tenth that for the heterocyclic amines. Of the other amines in urine there was no interference from the primary aliphatic amines—methylamine, ethylamine and iso-amylamine. Dimethylamine was, however, found to produce a colour similar to that of the heterocyclic amines, the sensitivity being almost identical for an equal weight of amine, but approximately twice as sensitive per mole in favour of the heterocyclic amines. Chromatography of the DNP derivatives showed that urinary dimethylamine content did not vary under the conditions of the experiments, whereas the heterocyclic amines were greatly increased by amino-acid ingestion. The method was therefore useful for assessing change in urinary piperidine plus pyrrolidine, but there was a constant blank value equivalent to an excretion of 0·7 to 1·3 mg. dimethylamine per hour in adults.

RESULTS

The results are summarized in Table I which, however, requires some explanation in the text. The first column gives the type of metabolic test, and the second the number of separate tests performed, as previously described in the 'methods' section. The incidence of diarrhoea is recorded in the third column. This was always unequivocal, the amino-acids either causing no change in the amount, frequency of voiding, or appearance of the faeces, or producing severe watery diarrhoea with voiding at half-hour intervals, and often accompanied by tenesmus and abdominal colic. The presence of part of the ingested amino-acid in faeces is recorded in column 4. The chromatographic technique used fails to distinguish lysine from ornithine, and it was therefore assumed that the appearance in the faeces of an amino-acid of R_f value identical to that given by mouth represented unabsorbed amino-acid (Fig. 2). Column 5 shows that the diamines were isolated by reineckate precipitation of faecal extracts more frequently in the cystinurics than in the normal controls. This correlates with the more frequent detection of the precursor amino-acids in the faeces of the cystinurics. Diamines were only found in the faeces of normal subjects on one occasion after lysine ingestion which caused intestinal hurry with watery diarrhoea. Fig. 3 shows a typical result from a normal subject and a cystinuric patient, cadaverine being detectable in the faeces of the patient only.

TABLE I

EFFECTS OF INGESTION OF L-LYSINE OR L-ORNITHINE MONOHYDROCHLORIDE IN FIVE HOMOZYGOUS CYSTINURIC PATIENTS AND 12 NORMAL CONTROLS

Type of Test	*No.*	*Diarrhoea*	*Administered Amino-acid Found in Faeces*	*Diamines in Faeces*	*Obvious Diaminuria*	*Suggestive Diaminuria*	*Evidence of Saturation of Jejunal Dibasic Amino-acid Transport System*	
							Arginine in Faeces	*Increase of Urinary Output of Both Piperidine and Pyrrolidine*
Cystinurics	4	Nil	4	4	1	2	4	4
Lysine			100%	100%	25%	50%	100%	100%
Cystinuric	1	Nil	1	nil	nil	nil	1	nil
Lysine + neomycin			100%				100%	
Cystinurics	2	2	2	2	nil	1	2	2
Ornithine		100%	100%	100%		50%	100%	100%
Normal subjects	10	2	2	1	nil	nil	2	nil
Lysine		20%	20%	10%			20%	
Normal subjects	1	Nil	1	nil	nil	nil	1	nil
Lysine + neomycin			100%				100%	
Normal subjects	2	1	1	nil	nil	nil	1	nil
Ornithine		50%	50%				50%	

Columns 6 and 7 give the incidence of diaminuria. 'Obvious diaminuria' means that a urine specimen was obtained which gave a dense precipitate when mixed with a saturated solution of ammonium reineckate, and the purified precipitate was identified as cadaverine reineckate. The methods used in identification were chromatography (Fig. 3) and preparation of dibenzoyl-cadaverine by a combination of the methods of Zaimis (1950) and Simon (1900). This dibenzoyl derivative was shown to be identical with pure dibenzoyl-cadaverine by three methods: determination of the melting point (135°C) both with and without admixture with the authentic compound, chromatography using butanol ethanol:2N ammonia, 7:1:2, as solvent, and by showing that the ultraviolet absorption spectrum was identical with that of the synthetic compound. 'Obvious diaminuria' is presumably comparable to that described by earlier workers and reviewed by Garrod (1908). The method is relatively insensitive as cadaverine must be in greater concentration than 0·2 mg./ml. before a reineckate precipitate is obtained. Diaminuria of this severity was found in only a single urine specimen obtained from a cystinuric eight to 10 hours after lysine ingestion. From the weight of reineckate obtained it could be calculated that 90 mg. cadaverine was excreted, corresponding to a conversion of 0·6% of ingested amino-acid to urinary diamine.

'Suggestive diaminuria' means that the colour and absorption spectrum of the solution obtained by the quantitative method for urinary amines was identical with that obtained from a mixture of cadaverine with either piperidine or pyrrolidine. The time relations of diaminuria are of importance. The specimen containing diamine was always a single two-hour collection obtained either two or four hours before the maximum output of heterocyclic amines, *i.e.*, from six to 10 hours after the first dose of amino-acid. This proves that diaminuria after lysine or ornithine ingestion in cystinuric patients is a transient phenomenon, and precedes maximum output of the heterocyclic amines, as would be predicted from the metabolic pathways shown in Fig. 1.

This paper provides evidence of impaired transport of dibasic amino-acids by the small intestine of cystinuric patients. This implies that these amino-acids share a common transport system, and that this would be more easily saturated by excess of a single dibasic amino-acid than would the transport system of a normal subject. We report two connected experimental observations supporting this view. Column 8 in Table I gives the more direct evidence. Ingestion of either lysine or ornithine by both normal subjects and cystinurics may impair the intestinal absorption of arginine, which is readily distinguished from the ingested amino-acids by its higher R_f value (Fig. 4) and by its reaction with the diacetyl-naphthol reagent for the guanidino-group. Arginine was recovered from the faeces in all tests in cystinuric patients, but in normal subjects only if there was intestinal hurry with watery diarrhoea or if intestinal transport had been temporarily impaired by pre-treatment with neomycin (Faloon, Fisher, and Duggan, 1958; Jacobson, Chodos, and Faloon, 1960). The last column of Table I contains more indirect evidence of saturation of intestinal transport, but is more conveniently described later together with the chromatograms of Figs. 8-10.

Figs. 2 and 3 give representative chromatographic results from which the data of Table I have been compiled. The effects of neomycin shown in Fig. 4 are of especial interest. This antibiotic has been shown to have a reversible toxic action on the gut mucosa involving impaired absorption of fats, carbohydrates, vitamins, and nitrogenous compounds (Faloon *et al.*, 1958; Jacobson *et al.*, 1960). Chromatography of faecal extracts after neomycin showed a gross increase of unabsorbed amino-acids, including lysine and arginine. Ingestion of lysine after neomycin in normal subjects and cystinurics causes an increased faecal output of both lysine and arginine. The damaged transport system of the healthy subject taking neomycin is thus more easily saturated than without the drug. There are two differences from the intestinal defect in cystinuric patients; the defect is generalized rather than specific for a limited number of dibasic amino-acids, and the effect of the antibiotic on the colonic flora prevents the formation of diamines and their metabolic products.

Fig. 5 gives the mean urinary output of heterocyclic amines (piperidine + pyrrolidine) after lysine in the 10 normal subjects and the four cystinuric patients. The maximum output occurs in both groups from 10 to 12 hours after the first dose of the amino-acid, but there is also a high excretion in the overnight specimen, 12 to 24 hours afterwards. The total excess output of heterocyclic amines in the cystinurics averaged 35·6 mg. (S.D. 12·7 mg.), and in the normal subjects 18·4 mg. (S.D. 10·4 mg.). There is considerable variation in the individual results as expected from a biological process dependent on intestinal absorption, bacterial putrefaction, and subsequent absorption and metabolism of the diamines produced. The two excretion figures are not in fact significantly different at the conventional 1 in 20 level of probability, the P value being 0·1 ($t = 1·7$). However, when the results of Figs. 5 and 7 are considered together, the difference is highly significant. The amount of

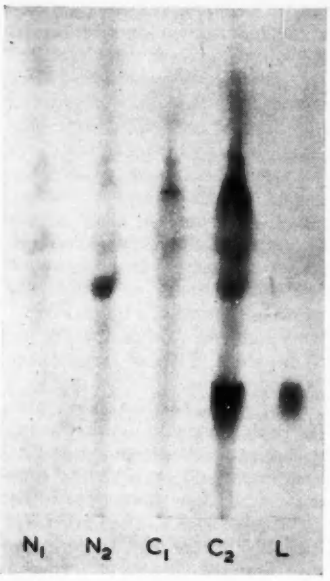

FIG. 2

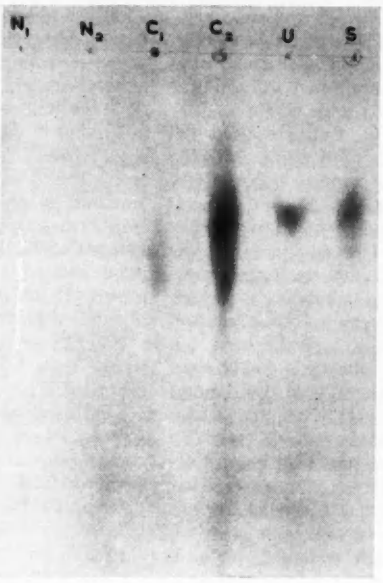

FIG. 3

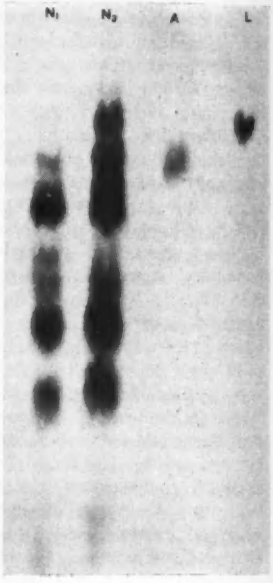

FIG. 4

FIG. 2. *Chromatograms of faecal extracts. N_1 = normal subject before lysine; N_2 = after lysine; C_1 = cystinuric before lysine; C_2 = after lysine; L = lysine marker. Butanol-acetic acid-water. Ninhydrin spray. There is only a slight increase of faecal lysine in the normal subject, but a great increase in the cystinuric.*

FIG. 3. *Chromatograms of amines in faecal extracts and urine after reineckate precipitation. N_1 = faecal extract of normal subject before lysine; N_2 = after lysine; C_1 = faecal extract of cystinuric before lysine; C_2 = after lysine; U = urine of cystinuric after lysine; S = cadaverine marker. Butanol-acetic acid-water. Ninhydrin spray. The faeces of the normal subject do not contain any detectable cadaverine either before or after lysine. There is some cadaverine in the faeces of the cystinuric before lysine and a great increase after ingestion of the amino-acid. Eight hours after lysine ingestion the cystinuric excreted considerable quantities of cadaverine in the urine.*

FIG. 4. *Chromatograms of faecal extracts. N_1 = normal subject taking neomycin before lysine ingestion; N_2 = after lysine; A = arginine marker; L = lysine marker. Butanol-acetic acid-water. Ninhydrin spray. The faeces of a normal subject taking neomycin contain considerable quantities of unabsorbed amino-acids. There is some lysine but a greater amount of arginine. After lysine ingestion there is increase of both lysine and arginine.*

amine excreted is equivalent to a conversion of 0·4% of the ingested lysine in the cystinuric patients and of 0·2% in the normal subjects. The latter conversion is equivalent to that from the normal intake of lysine + arginine contained in dietary proteins. From 6 to 9 g. of the amino-acids is ingested daily, and 3 to 20 mg. of piperidine plus pyrrolidine is excreted in the urine.

Fig. 6 gives the output of heterocyclic amines in the normal subject who showed the maximum increase of amine excretion. There is a considerable rise without neomycin ingestion, but no significant effect when taking the antibiotics. Chromatography showed there was, in fact, no heterocyclic amine excretion when taking neomycin (Fig. 9), the positive readings shown in Fig. 6 being due to urinary dimethylamine which is also estimated by the method. The true basal output of heterocyclic amines is given by the difference between the readings with and without neomycin. This difference is 0·63 mg./hour, a figure agreeing with von Euler's estimate (1945) of 3 to 20 mg. urinary piperidine plus pyrrolidine per day.

Fig. 7 gives the results after ornithine ingestion. Here the separation between the cystinurics and the normals is more obvious, but one of the patients received more amino-acid than the other three subjects. Ornithine hydrochloride is considerably

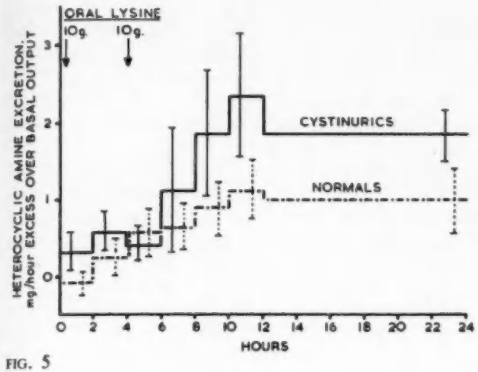

FIG. 5

FIG. 5. *Mean excretion or heterocyclic amines, expressed as mg./hour excess of basal output, in 10 normal subjects and four cystinurics after ingestion of 20 g. lysine. The vertical lines give the standard error of the means. There is considerable scatter and, although the cystinurics excrete about twice as much piperidine + pyrrolidine as compared to normal subjects, the difference is not statistically significant. In both normal subjects and in cystinuric patients the maximum output occurs from 10 to 12 hours after ingestion of the amino-acid.*

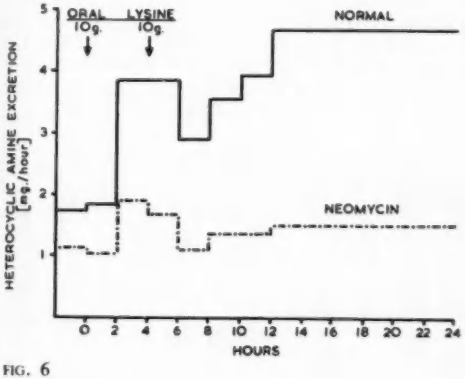

FIG. 6

FIG. 6. *Excretion of heterocyclic amines in the normal subject who showed the greatest amine output after lysine ingestion, with and without oral neomycin. There is no increase of urinary piperidine + pyrrolidine when taking neomycin, but a considerable rise when the antibiotic is not being taken. Chromatography while taking neomycin failed to show any urinary heterocyclic amine output. The values shown by the broken line represent the blank values of the method and are almost entirely accounted for by urinary dimethylamine.*

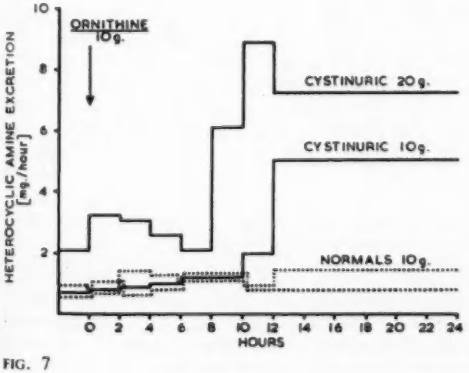

FIG. 7

FIG. 7. *Excretion of heterocyclic amines in two cystinurics and two normal subjects after ornithine ingestion. One cystinuric ingested 20 g. ornithine whereas the other three subjects were given 10 g. only. There is a great increase of urinary piperidine + pyrrolidine in the cystinurics but no rise in the two normal subjects. The maximal output in the cystinurics occurs 10 to 24 hours after amino-acid ingestion, and the amount of amines excreted is much greater than after lysine.*

more expensive than lysine hydrochloride and was found to be more toxic, accounting for the limited number of metabolic tests using this amino-acid. The excess heterocyclic amine output in the cystinurics was 88 mg. from 20 g. ornithine hydrochloride, and 57 mg. from 10 g. of the amino-acid, equivalent to conversion rates of 1% and 1·35% respectively. About three times as much amino-acid is converted to heterocyclic amines after ornithine as after lysine. This might be explained by the fact that lysine is a normal constituent of dietary protein, whereas ornithine is not. The two normal subjects taking ornithine showed no significant change in output of the heterocyclic amines.

The results given by the relatively less specific extraction procedure are confirmed and extended by the chromatographic results of Figs. 8-10. The use of DNP derivatives has the great advantage of distinguishing between piperidine, pyrrolidine, and dimethylamine, but is too time-consuming to have been applied to all 160 urine specimens analysed in this investigation. In practice the basal specimens and the samples showing high heterocyclic amine output 10 to 24 hours after amino-acid ingestion were analysed by this method. Great care was taken to ensure that specimens from the same patient were collected over equal time periods, and contained amounts of creatinine not differing by more than 10%. The urine of adult patients corresponding to about five minutes' excretion was a suitable quantity for analysis. By the chromatographic technique there are usually six distinct sorts of DNP derivatives of urinary amines and ammonia (Asatoor, 1960). Proceeding from the origin, the DNP derivatives are those of piperidine, pyrrolidine, ethylamine, dimethylamine, methylamine, and ammonia respectively. The fraction of DNP-ammonia extracted by cyclohexane is low compared to that of the DNP-amines, so that the ammonia spot is smaller than expected from the large amount of ammonia present in urine. The largest single spot in normal urine is usually that derived from dimethylamine. The procedures used in the tests did not have any effect on the output of the aliphatic amines, methylamine, ethylamine, or dimethylamine. Ammonia output was usually increased by the experimental procedure because the amino-acid hydrochlorides produce a systemic acidosis and a highly acid urine. The urinary excretion of the five amines was unaffected by alteration in acid-base

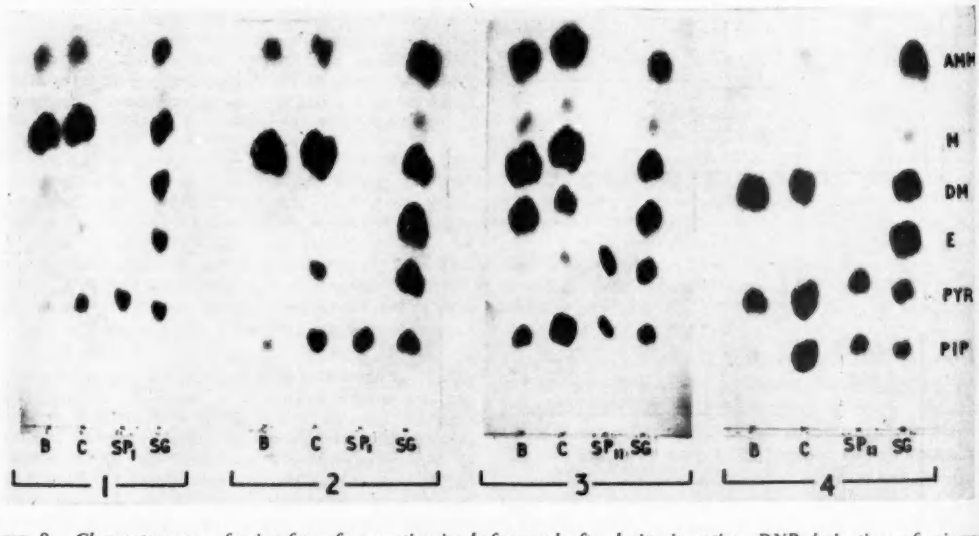

FIG. 8. *Chromatograms of urine from four cystinurics before and after lysine ingestion. DNP derivatives of urinary amines and ammonia extracted by cyclohexane. Reversed phase chromatography; chloroform:methanol:water as solvent. DNP derivatives photographed in ultra-violet light. B = before lysine ingestion. C = 10 to 12 hours after lysine ingestion. SG = general standard of the six DNP derivatives of piperidine (PIP), pyrrolidine (PYR), ethylamine (E), dimethylamine (DM), methylamine (M), and ammonia (AMM). SP_I = marker of DNP derivative of piperidine only; SP_{II} = mixed marker of DNP derivatives of piperidine and pyrrolidine. There is a great increase of piperidine excretion after lysine, but there is also some rise of pyrrolidine output. The four chromatograms (1, 2, 3, and 4) are grouped in order of the magnitude of the response, minimum increase of amine excretion on the left and maximum increase on the right.*

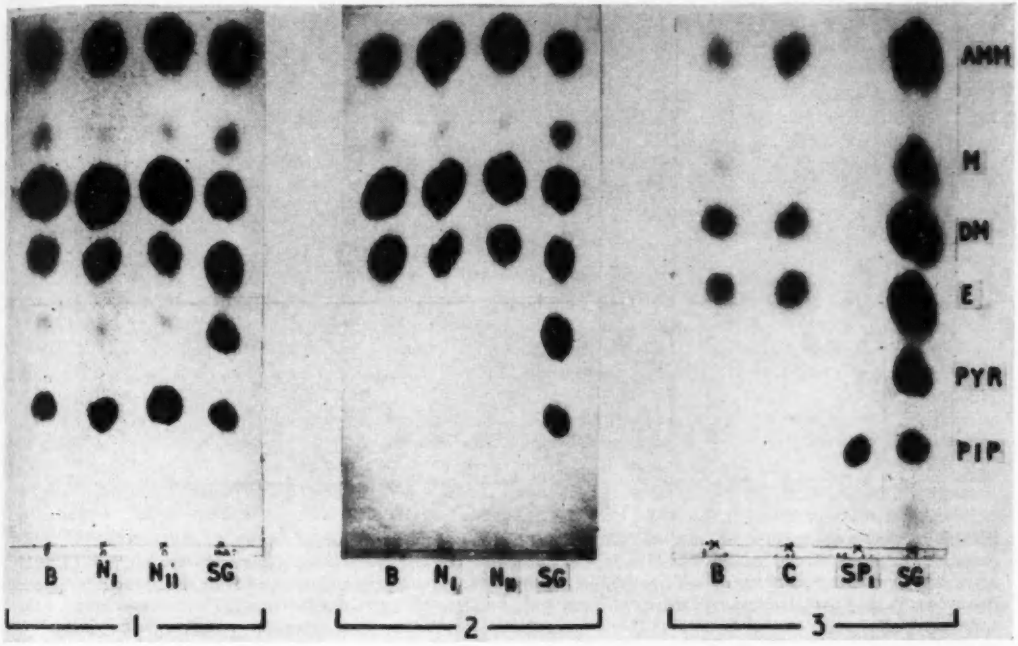

FIG. 9. *Chromatograms of urine from 1 = normal subject; 2 = normal subject pre-treated with neomycin; and 3 = a cystinuric patient pre-treated with neomycin, before and after lysine ingestion in all subjects. Technique as in Fig. 8. N_1 = 10 to 12 hours after the amino-acid; N_{11} = 12 to 24 hours after the amino-acid. Other symbols as in Fig. 8. In chromatogram 1 there is some rise of piperidine output after lysine, but no increase of pyrrolidine. In chromatograms 2 and 3 the oral neomycin has inhibited production and excretion of both piperidine and pyrrolidine but has not influenced the output of the aliphatic amines or of ammonia.*

balance. Changes in piperidine and pyrrolidine output are reported in detail.

Fig. 8 shows the chromatograms derived from the four cystinuric patients receiving lysine. They are classified in order of the magnitude of the effect produced, the one on the right labelled '4' showing the greatest rise of heterocyclic amines. In every case there was an increased output of both piperidine and pyrrolidine, although the change in the former amine was more obvious. This result was never found in a normal subject (Table I, last column). Here there was either no detectable change in amine output, or there was an increased excretion of piperidine only. The normal subject with the greatest effect is shown in Fig. 9, chromatogram 1. We interpret this difference between cystinurics and healthy controls as indicative of saturation of the intestinal transport mechanism of the cystinuric patients by a large dose of amino-acid, allowing dietary arginine to reach the colon and to be converted by bacteria to ornithine and then to putrescine (Fig. 1). Fig. 9 shows the effect of neomycin on heterocyclic amine output. Chromatograms 1 and 2 show the results obtained from the same normal subject with and without oral neomycin, and correspond to the quantitative analyses of Fig. 6. Chromatogram 3 gives the effect of neomycin in a cystinuric patient. In both cases the antibiotic causes complete disappearance of the piperidine and pyrrolidine spots, but has no effect on the other four spots. This proves that the urinary heterocyclic amines are completely dependent for their formation on bacterial action in the colon. Fig. 10 shows the results obtained from the four subjects after ornithine ingestion. In the cystinuric patients there is a great increase in the pyrrolidine spot and a smaller rise in the piperidine spot. This is again interpreted as evidence of saturation of the intestinal transport system. Excess ornithine causes both ingested ornithine and dietary lysine to reach the colon and to be converted both to cadaverine and putrescine. Ornithine ingestion by the normal

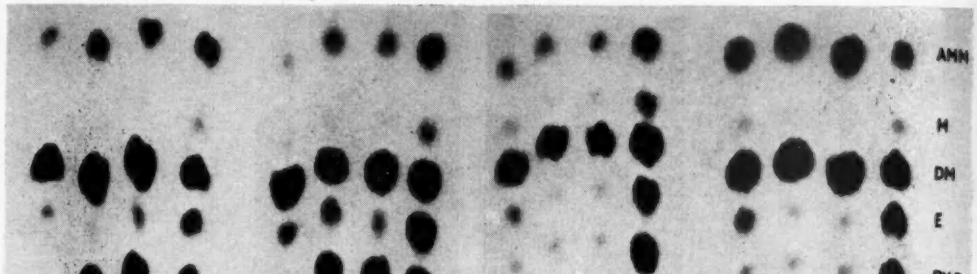

FIG. 10. *Chromatograms of urine from two cystinurics (1 and 2) and from two normal subjects (3 and 4) before and after ornithine ingestion (20 g. of the amino-acid in 1; 10 g. in the other three subjects). Technique as in Fig. 8. C_1 = 10 to 12 hours after ornithine ingestion; C_{11} = 12 to 24 hours after. Other symbols as in Figs. 8 and 9. In the cystinurics there is a great increase of pyrrolidine output after ornithine, and also some rise of piperidine output. There is no change in the excretion of the two heterocyclic amines in the normal subjects. In chromatogram 2 an extra spot, provisionally identified as the DNP derivative of iso-amylamine (IAM), has appeared below the piperidine spot after ornithine ingestion.*

subjects causes no appreciable change in heterocyclic amine output. In chromatogram 2 an extra spot was found after ornithine ingestion with a lower R_f value than DNP-piperidine. This was provisionally identified by chromatography as DNP-iso-amylamine. Further work is in progress in connexion with the precise identification of this abnormal urinary amine.

DISCUSSION

The results show that the metabolic effects of ingestion of a large dose of either L-lysine or L-ornithine hydrochloride in homozygous cystinuric patients differ from those in normal subjects. In cystinurics the amino-acids are incompletely absorbed in the small intestine, and can easily be detected in the faeces. This implies defective transport of the amino-acids by the jejunal cells similar to that previously known to occur in the proximal tubule cells of the kidney. The defect is not severe enough to cause any clinical disability or signs of malnutrition. Absorption of the involved amino-acids is almost complete at ordinary rates of ingestion, and therefore the loss of amino-acids in the faeces is not excessive on a normal diet. This is of especial importance in the case of lysine which is an essential amino-acid.

Ingestion of neomycin by normal subjects produces a broad spectrum malabsorption defect (Faloon *et al.*, 1958; Jacobson *et al.*, 1960) involving fats, carbohydrates, iron, vitamin B_{12}, and nitrogenous compounds, resembling the defect of idiopathic steatorrhoea. The present results show that the increase of faecal nitrogen after neomycin ingestion is partly due to rise in faecal amino-acids. Absorption of ingested lysine was defective both in the normal subjects treated with neomycin, and in homozygous cystinuric patients without neomycin. In both instances the ingested lysine saturated the intestinal transport system for dibasic amino-acids, and excess arginine as well as lysine appeared in the faeces. By contrast, in the majority of normal control subjects the transport system could not be fully saturated with the same dose of amino-acid, and neither lysine nor arginine appeared in the faeces.

Variation in the intestinal absorption of amino-acids can be classified in three functional grades:—

1 All the amino-acid is absorbed in the upper sterile portions of the gut and therefore there is no appreciable bacterial decomposition of unabsorbed amino-acid.

2 There is appreciable bacterial breakdown of unabsorbed amino-acid, but unchanged amino-acid is not excreted in greater amounts than the normal in the faeces.

3 There is still greater defective absorption with both increased bacterial breakdown, and loss of abnormal amounts of the amino-acid in faeces.

Obviously the boundary between 1 and 2 cannot be defined anatomically, because the level of the gut which first becomes contaminated with bacterial commensals varies from patient to patient, and at different times in the same subject. Decarboxylation of lysine, ornithine, and arginine to cadaverine, putrescine, and agmatine respectively (Fig. 1) is probably especially variable. Only certain bacteria, including *E. coli* and *Lactobacilli*, contain all three decarboxylases, *Proteus vulgaris* usually contains ornithine decarboxylase only, whilst none of the enzymes usually occur in *Strep. faecalis* and *Clostridia* (Gale, 1953). Decarboxylation is more active in an acid medium and pyridoxal phosphate is essential as a co-enzyme (Gale, 1940a). The decarboxylases are inducible enzymes, their concentration being increased by prolonged contact of the bacteria with the appropriate amino-acid substrate. Possibly the difference in diamine production by cystinurics and normals may partly be explained by induced excess of decarboxylase content of the colonic bacteria, as well as by increased concentration of amino-acid substrate in the colon. This explanation is especially likely in the ornithine experiments where one normal subject had equally severe watery diarrhoea as had the cystinuric patients, and large amounts of unabsorbed amino-acid were found in the stools. In the normal subject, however, putrescine was not detected in the faeces and there was no increase of excretion of heterocyclic amines. Further experiments involving bacterial metabolism will be necessary to solve this aspect of the problem. The present experiments would possibly have been even more conclusive if large doses of amino-acid had been administered over longer periods of time to produce maximal induction of bacterial decarboxylase. Such experiments in cystinurics might conceivably not be free from risk, as considerable amounts of potentially toxic amines were formed in the present milder experimental procedure.

After ingestion of large doses of lysine or ornithine by normal subjects there is either no increase of urinary amine output, or some increase of either piperidine or pyrrolidine alone, dependent on the amino-acid ingested. By contrast, in cystinurics there is an increase of both piperidine and of pyrrolidine, and occasionally excretion of unchanged diamine as well. Presumably if the experiments had been performed in starved cystinuric patients, there would have been increased output only of the heterocyclic amine corresponding to the particular amino-acid ingested. In fact, owing to the saturation of the jejunal transport system by a single ingested amino-acid, a mixture of lysine, arginine, and ornithine, partly derived from the diet, reached the colon, and therefore there was excess urinary output of both piperidine derived from lysine, and of pyrrolidine derived from arginine and ornithine.

The present experiments alone provide sufficient proof of a defective absorption and transport of lysine and ornithine by the jejunal cells of cystinuric patients. Strong supporting evidence is furnished by many previous observations which have been consistently misinterpreted. Von Udránszky and Baumann (1889) first reported excessive urinary and faecal output of the diamines, cadaverine and putrescine, in cystinuria. The daily urinary output of their patient ranged from 0·2 to 0·4 g. of derived benzoyldiamine, corresponding to an excretion of 60 to 120 mg. free diamine daily. In the present experiments the maximum output recorded after a large dose of precursor amino-acid was 90 mg. of cadaverine. Shortly afterwards, Stadthagen and Brieger (1889) detected cadaverine in the urine of two other cystinuric patients. Later work was reviewed by Garrod (1908) in his classical Croonian lectures on 'Inborn errors of metabolism'. Garrod stressed the inconstancy of diamine output in both faeces and urine in cystinuric patients. He recorded that diamines were isolated by nine later investigators between 1890 and 1908, but were not found by five other workers. His own personal experience included nine cases of cystinuria, and in only four of these patients were diamines detected in the urine, including one case with faecal diamine output also. In the case recorded by Cammidge and Garrod (1900) cadaverine was found in only two of 41 separate 24-hour urine specimens. The experiments of Loewy and Neuberg (1904) are of especial interest in relation to the present observations. Whilst there was no diaminuria at ordinary times in their case of cystinuria, the corresponding diamine appeared in the urine after ingestion of 5 g. of either lysine or arginine. Garrod and Hurtley (1906), repeating these experiments with arginine alone, failed to detect diamines in the urine of their patient. Garcia (1893) and Thiele (1907) found diaminuria in cystinurics to be increased by a high protein intake.

The former interpretation of these results is of great historical interest. Von Udránszky and Baumann (1889) considered that cystinuria was due to intestinal auto-intoxication with diamines or 'ptomaines'. Moreigne (1899) criticized this view because there was no clinical evidence of any chronic

intestinal abnormality. While agreeing that diamines were excreted in excess by cystinuric patients, he pointed out that excretion of indican and of phenols, derived from bacterial degradation of tryptophan and of phenylalanine or tyrosine respectively, was completely normal. In retrospect, this entirely correct observation should have suggested a selective rather than a general degradation of amino-acids within the colon. It was not then known, however, that decarboxylases capable of breaking down lysine, arginine, and ornithine were not present in body cells, and in fact only occurred in man within bacterial commensals in the colon. Moreigne therefore put forward the incorrect view, later supported by the authority of Garrod (1908), that the excess diamines were produced by the same unknown metabolic defect responsible for the increased cystine excretion. The true explanation of diaminuria in cystinurics was thus postponed for 60 years. In the decade after the elucidation of the renal transport defect in cystinuria by Dent and Rose (1951), the older observations of diaminuria have been dismissed as due to bacterial decomposition of excreted amino-acids in infected urine specimens. This possibility had, however, occurred to previous workers. Cammidge and Garrod (1900) and Hele (1909) were unable to find any increase of diamines in urine specimens from cystinurics after decomposition. In the present series of experiments only one of the patients had an urinary infection, and he did not excrete diamines. The urine and faecal specimens were immediately frozen after collection to prevent any appreciable bacterial decomposition outside the body. Diaminuria was always maximal six to 10 hours after amino-acid ingestion, whereas increased amino-acid excretion chiefly occurred in earlier urine collections, within the first six hours of the experiments.

In retrospect, the difficulties of earlier investigators of diaminuria in cystinurics were due to the inconstancy of the phenomenon. In the present experiments obvious diaminuria was found only in one of five patients, and was present for a short period of time. Previous workers have relied too much on analyses of 24-hour specimens of urine rather than on selected short-term collections. In the amino-acid loading experiments the doses used 50 years ago were too low to give a clear metabolic response. The amino-acids used in these older experiments had to be prepared by the investigators themselves, in contrast to the lavish commercial supply of the present day. After ingestion of lysine or ornithine by cystinuric patients, increased output of the heterocyclic amines, piperidine and pyrrolidine, is much more constant and predictable than is diaminuria. The metabolic pathway concerned was unknown before von Euler (1945) first reported the presence of piperidine in normal human urine.

The great variability of diamine excretion in cystinurics is now easily explained. A high output of diamines is dependent on the following favourable factors:—*a*, A high intake of lysine or arginine, whether as free amino-acids or as a high-protein diet; *b*, the presence in the colon of appropriate bacteria containing high concentrations of specific decarboxylases; *c*, a favourable acidic *p*H of colonic contents and free availability of pyridoxal phosphate as co-enzyme; *d*, a low body content of diamine oxidase which rapidly oxidizes absorbed diamines to the corresponding aldehydes.

Presumably diaminuria could be produced with more certainty by simultaneous administration of lysine or ornithine and a diamine oxidase inhibitor to a cystinuric, but such an experiment could not be guaranteed as free from risk to the patient.

In this series of experiments no observations were made of the intestinal absorption of cystine itself. Cystine and cysteic acid were not detected in chromatograms of faecal extracts after lysine or ornithine ingestion. Previous experiments (Brand, Cahill, and Harris, 1935; Brand, Cahill, and Block, 1935; Lewis, Brown, and White, 1936) have been consistently misinterpreted, but strongly support defective absorption of cystine by the gut of cystinuric patients as well as that of lysine, arginine, and ornithine. These investigators showed that ingestion of cystine, homocystine, and glutathione by cystinurics did not appreciably increase urinary cystine output, whereas methionine, cysteine, and homocysteine each caused a considerable rise. The results were incorrectly interpreted as due to an abnormality of metabolism of sulphur-containing amino-acids in cystinuria. In more recent years these important results have been attributed to the relative insolubility of cystine in the gut contents reducing the speed of cystine absorption (Dent, Heathcote, and Joron, 1954a; Dent, Senior, and Walshe, 1954b). This explanation is regarded as improbable for three reasons. It would imply a poor absorption of cystine by normal healthy subjects as well as by cystinurics, an unlikely hypothesis for an amino-acid which accounts for about 2% of the amino-nitrogen of protein foods. Homocystine is considerably more soluble than cystine (Milne, 1961) but both amino-acids are poorly absorbed by cystinurics. Neil (1959), studying absorption of sulphur-containing amino-acids in the rat's intestine, found that L-cystine was absorbed at about 70% the speed of L-cysteine, a variation which would not explain the gross difference in the effects of these two amino-acids in cystinurics. Lack of solubility would, in fact, only reduce the speed of cystine

absorption in the gut if the amino-acid was in sufficient concentration to crystallize out within the gut lumen.

The results are more adequately explained by the present concept of a specific intestinal transport defect of dibasic amino-acids similar to the known renal tubular transport defect. Homocystine is structurally similar to cystine, both amino-acids possessing two amino-groups in each molecule. It would therefore be expected to be absorbed from the intestine less rapidly in cystinuric patients than in normal subjects. By contrast, methionine, cysteine, and homocysteine each possess a single amino-group per molecule and would be expected to be absorbed normally. Glutathione, a tripeptide containing cysteine, is a very labile compound and has a considerable lower redox potential than cysteine itself (Calvin, 1954). It is therefore possibly oxidized to the di-sulphur compound within the gut lumen, and this would form cystine after hydrolysis by gut peptidases.

Dent *et al.* (1954a and b) repeated Brand's work except that they analysed plasma concentration of cystine by polarographic and microbiological assays as well as measuring cystine output in the urine. At this time the view of Dent and Rose (1951) that there was a specific renal transport defect in cystinuria had not been universally accepted, and therefore the discussion in these papers is chiefly concerned with the urinary aspect of the disease. It has proved profitable to re-examine the results of Dent *et al.* (1954a and b) in connexion with our more general view of the transport abnormality. The results of plasma levels of cystine have been recalculated as mean increments of the basal value after ingestion of similar amounts of L- and DL-cystine, L-cysteine, and DL-methionine by cystinurics and normal subjects (Fig. 11). It is seen that after ingestion of L- and DL-cystine, plasma levels of cystine do not increase as much in cystinurics as in normal subjects. This is readily explained by an intestinal transport defect which, by analogy with the results obtained in Hartnup disease (Milne *et al.*, 1960), would be expected to involve transport of the D-enantiomorph as well as the natural L-amino-acid. By contrast, plasma levels of cystine after ingestion of L-cysteine and DL-methionine increase more in cystinurics than in normal subjects (Fig. 11). This cannot be explained by an intestinal transport defect, but would be predicted if it is assumed that the defect is more generalized and involves transport of the amino-acids from the extracellular to the intracellular compartment, especially in the tissue of greatest quantitative importance, voluntary muscle. Our view is that cysteine and methionine are absorbed from the gut at equal rates in cystinurics

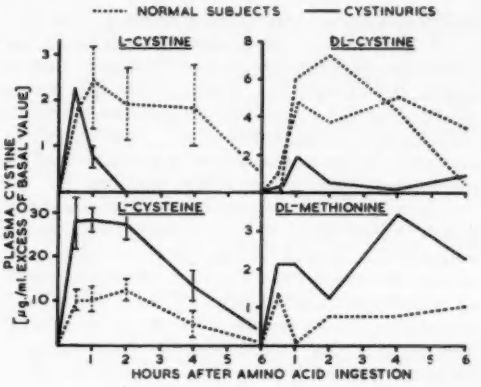

FIG. 11. *Mean increments of plasma cystine after ingestion of 5·0 g. L-cystine (upper left), 5·0 g. DL-cystine (upper right), 6·0 g. L-cysteine HCl (lower left), and 6·2 g. DL-methionine (lower right) respectively. The vertical lines give the standard errors of the means in the case of L-cystine and L-cysteine. Only three observations were made in the case of DL-cystine; the results in the case of DL-methionine are not statistically significant. After L-cystine and DL-cystine the plasma levels of cystine increase more in the normal subjects than in the cystinurics but the reverse occurs after L-cysteine and DL-methionine. The data are calculated from the results of Dent* et al. *(1954a and b).*

and in normal subjects, and are rapidly metabolized to cystine, conversion being greater in the case of cysteine. The cystine formed is transferred from the blood and extracellular fluid into the intracellular space, especially of voluntary muscle, at a slower rate in the cystinurics, thus accounting for the observed higher plasma values. Obviously this hypothesis requires further investigation, and could easily be settled by following the rate of disappearance of cystine, preferably labelled with ^{35}S, from the plasma after intravenous injection in cystinurics and normal subjects. Becker and Green (1958) attempted to investigate this aspect of the disease by studies of the transfer of labelled cystine and lysine *in vitro* into leucocytes obtained from cystinuric patients. The entry into cells was, however, found to be identical to that into leucocytes from normal subjects.

The isolated defect of cystinuria suggests that transport of the dibasic amino-acids is completely separate from that of the monoamino-monocarboxylic amino-acids involved in the related condition, Hartnup disease. This view is supported by results in the experimental animal. Lysine and arginine cannot be observed from the gut of the rat against a transport gradient as can the

monoamino-monocarboxylic amino-acids (Wiseman, 1955). This, however, does not necessarily prove that absorption proceeds by passive diffusion, and in fact Di Bella (1960) has recently shown that during absorption of lysine from the gut of the rat, there is concentration of the amino-acid within the wall of the gut to values above that of the contents of the gut lumen. The amino-acids involved in the transport defect of cystinuria are all concentrated within the intracellular fluid of cells from human liver and conjunctiva, and carcinoma cells in tissue culture to a much less extent that the monoamino-monocarboxylic amino-acids (Piez and Eagle, 1958). The dibasic amino-acids all show mutual competitition for the proximal renal tubular transport system (Robson and Rose, 1957), similar to their competition for intestinal transport.

There is no evidence to date that the intestinal defect of cystinuria is of more than theoretical importance. In Hartnup disease, the intestinal absorption defect is clinically of greater importance than the renal transport defect in causing the signs and symptoms of the disorder (Milne *et al.*, 1960; Jepson and Spiro, 1960). The reverse applies in cystinuria. Repeated ingestion of large doses of lysine, arginine, or ornithine might be dangerous in cystinuric patients. Lysine hydrochloride, at a dosage of up to 40 g. daily, has recently been advocated (Brailovsky, Silva, Del Campo, and Marusic, 1959; Rubin, Spritz, Mead, Herrmann, Braveman, and Luckey, 1960; Lasser, Schoenfeld, and Friedberg, 1960) as a source of chloride in order to potentiate the action of mercurial diuretics in the treatment of refractory oedema. The possible adverse pharmacological actions of large doses of lysine have received little attention. This paper shows that considerable quantities of lysine at high dosage may escape absorption in the small intestine, and be converted by bacteria in the colon into toxic diamines and then to heterocyclic amines. Piperidine has toxic actions resembling those of the alkaloid coniine (n-propylpiperidine), but is less poisonous than the plant alkaloid (Moore and Row, 1898). The diamines are powerful convulsants when given in high dosage to the experimental animal. Lysine toxicity would probably increase on repeated administration from induction of lysine decarboxylase within colonic bacteria. The risk of toxicity would obviously be greater in cystinuric patients. Simultaneous neomycin would, however, prevent the formation of toxic amines by sterilization of colonic contents.

SUMMARY

The effects of ingestion of large doses of lysine and ornithine hydrochlorides are compared in homozygous cystinuric patients and in normal control subjects. The amino-acids were less completely absorbed by the patients and a considerable fraction appeared unchanged in the faeces. By contrast, in normal subjects absorption was complete except in three individuals who had intestinal hurry with watery diarrhoea. Neomycin reduces amino-acid absorption both before and after large doses of lysine.

In cystinuric patients, but not in normal subjects, ingestion of lysine and ornithine increases the faecal output of arginine, suggesting saturation of a common intestinal transport system. The unabsorbed amino-acids are converted by colonic bacteria to the diamines, cadaverine and putrescine. These are absorbed from the colon in cystinuric patients and may occasionally occur in sufficient concentration to be easily detectable in the urine. The greater fraction of absorbed diamine is oxidized by diamine oxidase, with partial conversion to the heterocyclic amines, piperidine and pyrrolidine, which are promptly excreted in the urine. Output of the heterocyclic amines is greater in cystinuric patients after lysine or ornithine ingestion than in normal subjects. Owing to saturation of the intestinal transport system for dibasic amino-acids, lysine and ornithine ingestion in cystinurics causes increased output of both piperidine and pyrrolidine, whereas only the single heterocyclic amine corresponding to the ingested amino-acid rises in normal subjects.

The results suggest that the amino-acid transport defect known to occur in the kidney of cystinurics is also present in the gut. This view of the disease explains many apparently anomalous results obtained by previous investigators.

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REFERENCES

Asatoor, A. M. (1960). Paper chromatography of 2, 4-dinitrophenyl derivatives of amines. *J. Chromatography*, **4**, 144-152.

——, and Kerr, D. N. S. (1961). Amines in blood and urine in relation to liver disease. *Clin. chim. Acta*, **6**, 149-156.

——, and Milne, M. D. (1961). Unpublished observations.

Baron, D. N., Dent, C. E., Harris, H., Hart, E. W., and Jepson, J. B. (1956). Hereditary pellagra-like skin rash with temporary cerebellar ataxia, constant renal amino-aciduria and other bizarre biochemical features. *Lancet*, **2**, 421-428.

Becker, F. F., and Green, H. (1958). Incorporation of cystine and lysine by normal and 'cystinuric' leukocytes. *Proc. Soc. exp. Biol. (N.Y.)*, **99**, 694-696.

Brailovsky, D., Silva, C., Del Campo, E., and Marusic, E. (1959). The use of L-lysine monohydrochloride in the treatment of mercurial resistant edema. *Amer. J. med. Sci.*, **238**, 287-296.

Brand, E., Cahill, G. F., and Block, R. J. (1935). Cystinuria IV. The metabolism of homocysteine and homocystine. *J. biol. Chem.*, **110**, 399-410.

——, ——, and Harris, M. M. (1935). Cystinuria. II. The metabolism of cystine, cysteine, methionine, and glutathione. *Ibid.*, **109**, 69-83.

Calvin, M. (1954). Mercaptans and disulfides: some physics, chemistry and speculation. In *Glutathione, a Symposium*, pp. 3-30. Ed. Colowick, S., Lazarow, A., Racker, E., Schwarz, D. R., Stadtman, E., and Waelsch, H. Academic Press, New York.

Cammidge, P. J., and Garrod, A. E. (1900). On the excretion of diamines in cystinuria. *J. Path. Bact.*, **6**, 327-333.

Dent, C. E., and Harris, H. (1951). The genetics of 'cystinuria'. *Ann. Eugen.* (*Lond.*), **16**, 60-87.

——, Heathcote, J. G., and Joron, G. E. (1954a). The pathogenesis of cystinuria. I. Chromatographic and microbiological studies of the metabolism of sulphur-containing amino-acids. *J. clin. Invest.*, **33**, 1210-1215.

——, and Rose G. A. (1951). Amino-acid metabolism in cystinuria. *Quart. J. Med.*, n.s., **20**, 205-219.

——, Senior, B., and Walshe, J. M. (1954b). The pathogenesis of cystinuria. II. Polarographic studies of the metabolism of sulphur-containing amino-acids. *J. clin. Invest.*, **33**, 1216-1226.

Di Bella, S. (1960). Absorption of L-lysine in the small intestine of rats. *Experientia* (*Basel*), **16**, 367-368.

Euler, U. S. von (1945). The occurrence and determination of piperidine in human and animal urine. *Acta. pharmacol.* (*Kbh.*), **1**, 29-59.

Faloon, W. W., Fisher, C. J., and Duggan, K. C. (1958). Occurrence of a sprue-like syndrome during neomycin therapy. *J. clin. Invest.*, **37**, 893.

Gale, E. F. (1940a). The production of amines by bacteria. I. The decarboxylation of amino-acids by strains of *Bacterium coli*. *Biochem. J.*, **34**, 392-413.

——. (1940b). The production of amines by bacteria. 3. The production of putrescine from *L*(+)-arginine by *Bacterium coli* in symbiosis with *Streptococcus faecalis*. *Ibid.*, **34**, 853-857.

——. (1953). Amino-acid decarboxylase. *Brit. med. Bull.*, **9**, 135-138.

Garcia, S. A. (1893). Ueber Ptomaine. *Hoppe-Seylers Z. physiol. Chem.*, **17**, 577-595.

Garrod, A. E. (1908). The Croonian lectures on inborn errors of metabolism. *Lancet*, **2**, 142-148 and 214-220.

——, and Hurtley, W. H. (1906). Concerning cystinuria. *J. Physiol.* (*Lond.*), **34**, 217-223.

Harris, H., Mittwoch, U., Robson, E. B., and Warren, F. L. (1955a). The pattern of amino-acid excretion in cystinuria. *Ann. hum. Genet.*, **19**, 196-208.

——, ——, ——, ——, (1955b). Phenotypes and genotypes in cystinuria. *Ibid.*, **20**, 57-91.

——, and Robson, E. B. (1955). Variation in homozygous cystinuria. *Acta genet.* (*Basel*), **5**, 381-390.

——, and Warren, F. L. (1953). Quantitative studies on the urinary cystine in patients with cystine stone formation and in their relatives. *Ann. Eugen.* (*Lond.*), **18**, 125-171.

Hele, T. S. (1909). Metabolism in cystinuria. *J. Physiol.* (*Lond.*), **39**, 52-72.

Jacobson, E. D., Chodos, R. B., and Faloon, W. W. (1960). An experimental malabsorption syndrome induced by neomycin. *Amer. J. Med.*, **28**, 524-533.

Jepson, J. B., and Spiro, M. J. (1960). Hartnup disease. In *The Metabolic Basis of Inherited Disease*, pp. 1338-1364. Ed. Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S. McGraw-Hill, New York.

Krebs, H. A., and Henseleit, K. (1932). Untersuchungen über die Harnstoffbildung im Tierkörper. *Hoppe-Seylers Z. physiol. Chem.*, **210**, 33-66.

Lasser, R. P., Schoenfeld, M. R., and Friedberg, C. K. (1960). L-lysine monohydrochloride. A clinical study of its action as a chloruretic acidifying adjuvant to mercurial diuretics. *New Engl. J. Med.*, **263**, 728-733.

Lewis, H. B., Brown, B. H., and White, F. R. (1936). The metabolism of sulfur. 23. The influence of the ingestion of cystine, cysteine, and methionine on the excretion of cystine in cystinuria. *J. biol. Chem.*, **114**, 171-184.

Loewy, A., and Neuberg, C. (1904). Über Cystinurie. *Hoppe-Seylers Z. physiol. Chem.*, **43**, 338-354.

Mann, P. J. G., and Smithies, W. R. (1955). Plant enzyme reactions leading to the formation of heterocyclic compounds. I. The formation of unsaturated pyrrolidine and piperidine compounds. *Biochem. J.*, **61**, 89-100.

Milne, M. D. (1961). Unpublished observations.

——, Crawford, M. A., Girão, C. B., and Loughridge, L. W. (1960). The metabolic disorder in Hartnup disease. *Quart. J. Med.*, n.s., **29**, 407-421.

Moore, B., and Row, R. (1898). A comparison of the physiological actions and chemical constitution of piperidine, coniine and nicotine. *J. Physiol.* (*Lond.*), **22**, 273-295.

Moreigne, H. (1899). Étude sur la cystinurie. *Arch. Méd. exp.*, **11**, 254-312.

Neil, M. W. (1959). The absorption of cystine and cysteine from rat small intestine. *Biochem. J.*, **71**, 118-124.

Piez, K. A., and Eagle, H. (1958). The free amino acid pool of cultured human cells. *J. biol. Chem.*, **231**, 533-545.

Robson, E. B., and Rose, G. A. (1957). The effect of intravenous lysine on the renal clearances of cystine, arginine and ornithine in normal subjects, in patients with cystinuria and Fanconi syndrome and in their relatives. *Clin. Sci.*, **16**, 75-93.

Rothstein, M., and Miller, L. L. (1954). The conversion of lysine to pipecolic acid in the rat. *J. biol. Chem.*, **211**, 851-858.

Rubin, A. L., Spritz, N., Mead, A. W., Herrmann, R. A., Braveman, W. S., and Luckey, E. H. (1960). The use of L-lysine monohydrochloride in combination with mercurial diuretics in the treatment of refractory fluid retention. *Circulation*, **21**, 332-336.

Simon, C. E. (1900). Cystinuria and its relation to diaminuria. *Amer. J. med. Sci.*, **119**, 39-54.

Stadthagen, M., and Brieger, L. (1889). Ueber Cystinurie. *Berl. klin. Wschr.*, **26**, 344-347.

Strecker, H. J. (1960). The interconversion of glutamic acid and proline. III. Δ_1-pyrroline-5-carboxylic acid dehydrogenase. *J. biol. Chem.*, **235**, 3218-3223.

Suzuki, T., and Hagihara, F. (1954). Conversion of cadaverine to piperidine by hog kidney homogenate. *J. pharm. Soc. Japan*, **74**, 546-547.

Thiele, F. H. (1907). Concerning cystinuria and diamines. *J. Physiol.* (*Lond.*), **36**, 68-80.

Udránszky, L. von, and Baumann, E. (1889). Ueber das Vorkommen von Diaminen, sogenannten Ptomainen, bei Cystinurie. *Hoppe-Seylers Z. physiol. Chem.*, **13**, 562-594.

Wiseman, G. (1955). Preferential transference of amino-acids from amino-acid mixtures by sacs of everted small intestine of the golden hamster (*mesocricetus auratus*). *J. Physiol.* (*Lond.*), **127**, 414-422.

Wollaston, W. H. (1810). On cystic oxide, a new species of urinary calculus. *Phil. Trans.*, **100**, 223-230.

Zaimis, E. J. (1950). The synthesis of methonium compounds, their isolation from urine, and their photometric determination. *Brit. J. Pharmacol.*, **5**, 424-430.

Gut, 1961, **2**, 338

The effect of corticotrophin and corticosteroids on the external secretion of the pancreas in dogs

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SYNOPSIS In acute experiments in dogs cortisone and hydrocortisone increase the concentration and output of bicarbonate in pancreatic secretion whereas prolonged administration of these hormones leads to a diminished bicarbonate response. In both acute and chronic experiments, however, cortisone and hydrocortisone produce an increased output of the enzymes amylase and trypsin.

There are several published reports of the effect of corticotrophin and corticosteroids on gastric secretion in man and other species but only two, both in man, concerning their effect upon pancreatic secretion. Pfeffer and Hinton (1956) stimulated the pancreas with intravenous secretin and insulin after prior injection or infusion of hydrocortisone and corticotrophin. Dreiling, Janowitz, and Rolbin (1958) injected secretin, collected the duodenal contents for a subsequent period of 80 minutes and then, after injecting either corticotrophin, hydrocortisone, or prednisolone, continued duodenal aspiration for a further five hours. Amylase was the only enzyme determined in each study. In another paper Dreiling and Janowitz (1959) further analysed the data from their original work. Both groups of workers concluded that corticotrophin and corticosteroids depressed the production of bicarbonate and Dreiling *et al.* found a similar effect on amylase. The present study was begun for two reasons: first, to examine the effects of corticotrophin and corticosteroids on pancreatic secretion under more exact conditions in the dog, and secondly, to gain more knowledge concerning the degree of tissue specificity involved in such actions of corticosteroids as the known one on gastric secretion.

The mode of action of corticotrophin and corticosteroids on the parietal cells of the stomach, or, indeed, on any glandular tissue, is uncertain. There is evidence that steroids may affect the d-amino acid oxidase enzyme involved in intracellular processes of oxidation and reduction (Hayano and Dorfman, 1951; Umbreit, 1951). It is arguable that if the action on gastric secretion is effected by influencing intracellular metabolism then it should be as readily demonstrable in other glandular structures of the alimentary tract with exocrine function, including the pancreas.

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METHOD

Two dogs were prepared for investigation of the effects of long-term administration of hormones by the formation of an exteriorized loop of duodenum. At operation the pancreatic accessory duct was found and divided between ligatures. The duodenum was transected immediately proximal to the main pancreatic duct and the proximal portion anastomosed, end to side, to the fourth part of the duodenum to restore alimentary continuity. The free end distal to the transection was closed and brought out of the abdominal cavity and permanently exteriorized outside the recti muscles which were closed together in the usual manner. A previously prepared skin flap was then sewn around the exteriorized duodenal loop to form a 'suitcase handle'. A silver cannula with a screw cap was inserted into the anti-mesenteric border of the loop immediately opposite the main pancreatic duct (Fig. 1). Pancreatic secretion was subsequently collected by placing a non-traumatic clamp across the skin-covered loop immediately distal to the cannula. A small closed chamber of about 2 cm. diameter was thus formed around the main duct, and, in the erect position of the dog, uncontaminated pancreatic juice flowed down the cannula from which the cap had been unscrewed.

In three other dogs after likewise dividing the accessory pancreatic duct a double or jacketed silver cannula (Fig. 2) was inserted into the duodenum opposite to the main duct opening. The duodenum was not otherwise disturbed. Recovery of pancreatic juice was obtained by advancing the inner tube until the conical flange at the

FIG. 1

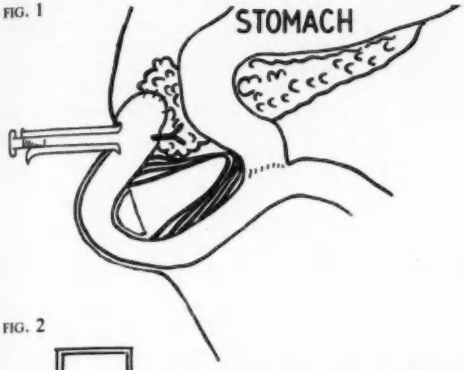

FIG. 2

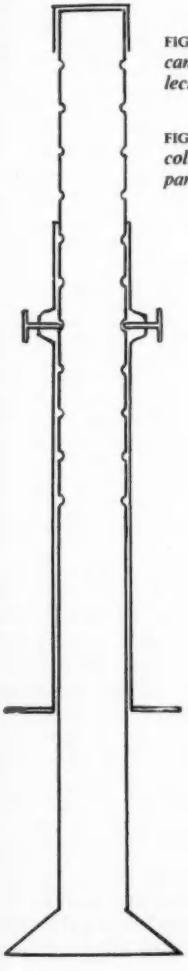

FIG. 1. *Skin-covered, exteriorized, cannulated duodenal loop for collecting pancreatic secretion.*

FIG. 2. *A double cannula for direct collection of secretion from the main pancreatic duct.*

end impinged on the duodenal wall around the main duct opening and thus isolated it.

In all experiments the pancreatic secretion was collected under glycerol and in a container surrounded by ice.

Amylase was determined by a modification of the method of Lagerlöf (1942), trypsin according to the method of Gowenlock (1953) and expressed as Gowenlock units, and lipase by the method described by Sammons, Frazer, and Thompson (1956), using olive oil as the substrate.

PROCEDURES

EFFECT OF LONG-TERM ADMINISTRATION OF CORTISONE Two dogs were used and six control experiments made on each to determine the normal response to the intravenous injection of secretin and pancreozymin.

Cortisone (cortisone acetate, Glaxo), either 25 or 50 mg., was given orally each day for 53 days to one dog and for 120 days to the other, and the test procedure repeated at intervals. Secretin and pancreozymin (Boots) were given intravenously in a dose of 2 units/kg. body weight. This dose was found to produce the maximum response from the pancreas under normal conditions. The design of the experiment was to collect fasting pancreatic juice for 15 minutes, then secretin was injected, and the response collected for 30 minutes. At the end of that time the pancreozymin was injected and the secretion collected for a further 30 minutes.

EFFECT OF LONG-TERM ADMINISTRATION OF CORTICOTROPHIN This study was carried out on one dog. Control experiments were made to establish the pancreatic secretory response to a standardized meal and to secretin and pancreozymin.

Eight ounces of a proprietary food (Lassie) were fed and pancreatic secretion continuously collected for three hours. Tins of food from the same batch of manufacture were used throughout.

The stimulating procedures were repeated at intervals after beginning the daily intramuscular injection of 40 units of corticotrophin.

A capsule containing 1 g. of potassium chloride was fed each day to the dogs in both groups.

EFFECT OF SINGLE INJECTIONS OF HYDROCORTISONE Three dogs were used to test the effect of single injections of hydrocortisone on the pancreatic response to secretin and pancreozymin.

Injection of hydrocortisone during continuous infusion of hormones Secretin or pancreozymin was infused intravenously using a motor-driven continuous infusion apparatus (Palmer). When the volume output of the pancreatic response had reached a plateau, 100 mg. of hydrocortisone hemisuccinate sodium (Glaxo) was injected intravenously.

Administration of hydrocortisone before injection of secretin and pancreozymin One hundred milligrams of hydrocortisone hemisuccinate sodium were injected intravenously either 15 or 30 or 60 minutes before the intravenous injection of secretin and pancreozymin in

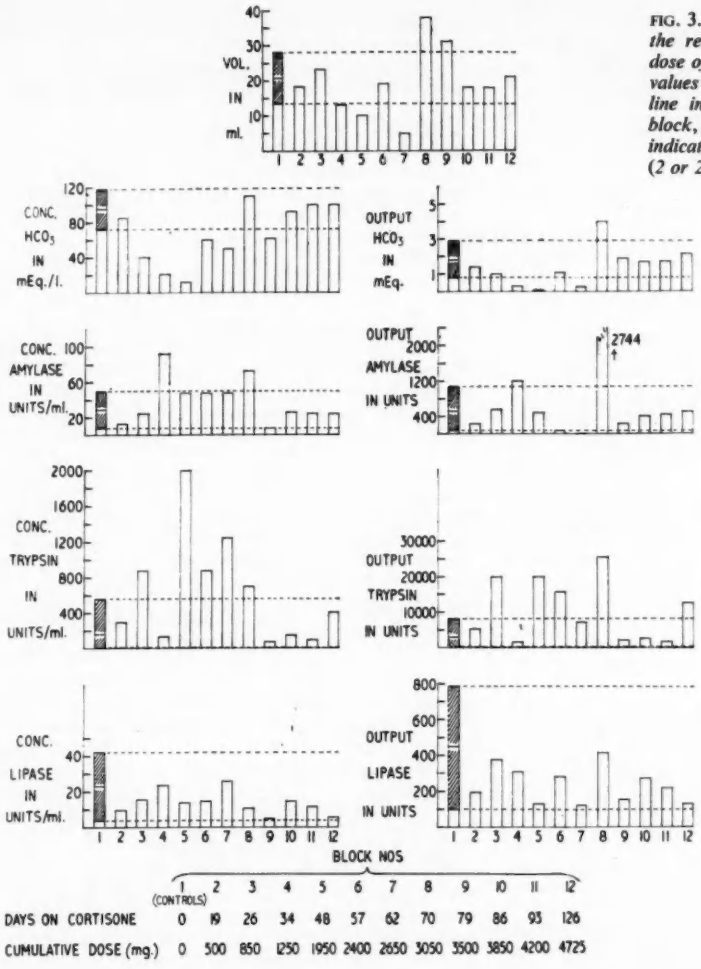
VOL. IN ml.
CONC. HCO_3 IN mEq./l.
OUTPUT HCO_3 IN mEq.
CONC. AMYLASE IN UNITS/ml.
OUTPUT AMYLASE IN UNITS
2744
CONC. TRYPSIN IN UNITS/ml.
OUTPUT TRYPSIN IN UNITS
CONC. LIPASE IN UNITS/ml.
OUTPUT LIPASE IN UNITS
BLOCK NOS
(CONTROLS)
DAYS ON CORTISONE 0 19 26 34 48 57 62 70 79 86 93 126
CUMULATIVE DOSE (mg.) 0 500 850 1250 1950 2400 2650 3050 3500 3850 4200 4725

FIG. 3. *Dog F. The effect of cortisone on the response to a standard intravenous dose of secretin. The mean of the control values is represented by the horizontal line interrupting the cross-hatched first block, and the extent of the cross-hatching indicates the 1 in 40 confidence limits (2 or 2·5% probability value).*

the standard test detailed above and the results compared with control experiments, omitting the hydrocortisone.

RESULTS

In the text, results are only considered to be significant when they differ from the control values by more than two standard deviations.

EFFECT OF PROLONGED ADMINISTRATION OF CORTISONE ON RESPONSE TO SECRETIN In dog F there were considerable variations in the results over the whole period of cortisone administration but a striking difference appeared between the effects on electrolytes and enzymes. The concentration and output of bicarbonate tended to fall whereas the concentration and output of trypsin tended to rise. These effects were most evident during the second and third months of administration. Significant increases in volume appeared episodically and in two tests the concentration and output of amylase were raised. The concentration and output of lipase were unaffected throughout (Fig. 3).

In dog E, only four experiments were made after beginning the administration of cortisone. The output of bicarbonate was again found to be reduced but no significant changes appeared in the concentration and output of the enzymes by the fifty-third day, soon after which the dog died from

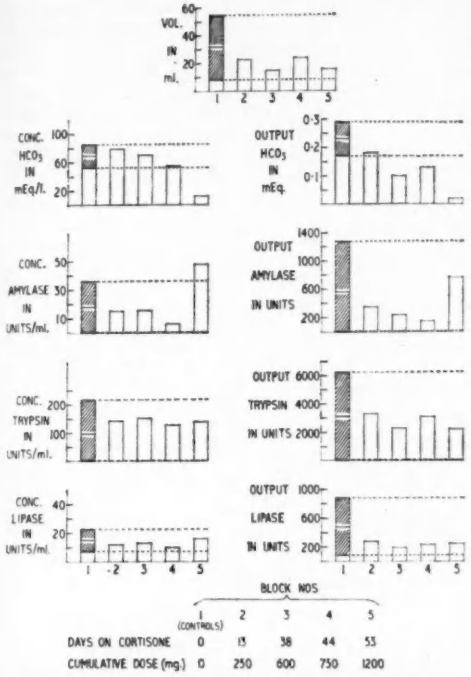
VOL. IN ml.
CONC. HCO3 IN mEq/l.
OUTPUT HCO3 IN mEq.
CONC. AMYLASE IN UNITS/ml.
OUTPUT AMYLASE IN UNITS
CONC. TRYPSIN IN UNITS/ml.
OUTPUT TRYPSIN IN UNITS
CONC. LIPASE IN UNITS/ml.
OUTPUT LIPASE IN UNITS
BLOCK NOS
1 (CONTROLS) 2 3 4 5
DAYS ON CORTISONE 0 15 38 44 53
CUMULATIVE DOSE (mg.) 0 250 600 750 1200

FIG. 4. *Dog E. The effect of cortisone on the response to a standard intravenous dose of secretin. The mean of the control values is represented by the horizontal line interrupting the cross-hatched first block, and the extent of the cross-hatching indicates the 1 in 40 confidence limits (2 or 2·5% probability value).*

a perforation of the duodenum caused by pressure necrosis from the cannula (Fig. 4).

EFFECT OF PROLONGED ADMINISTRATION OF CORTISONE ON RESPONSE TO PANCREOZYMIN In dog F, there again appeared significant effects upon the bicarbonate and trypsin components of the secretion and also upon the volume output.

The volume was raised in the second and third months of the tests, as was both concentration and output of trypsin. Although the concentration of bicarbonate was lowered in many tests the increase in volume output caused an increase in bicarbonate output on four occasions. Furthermore in most of the tests the output of amylase was significantly raised.

The output of lipase was unaffected in all but two tests, in which it was raised, as a result of increases in volume output (Fig. 5).

In dog E, the only significant changes in the few available results show a reduction in concentration and output of bicarbonate and a rise in concentration and output of amylase and trypsin (Fig. 6). There were no changes noted in the lipase response.

EFFECT OF PROLONGED ADMINISTRATION OF CORTICOTROPHIN IN RESPONSE TO A MEAT MEAL The volume output was significantly reduced after the administration of corticotrophin. Changes otherwise appeared only in the bicarbonate response in which the concentration was reduced in half the experiments and the output in all. No significant changes were noted in the concentrations and outputs of the three enzymes (Fig. 7).

EFFECT OF PROLONGED ADMINISTRATION OF CORTICOTROPHIN ON RESPONSE TO SECRETIN AND PANCREOZYMIN The results of only three experiments were available and demonstrated a reduction in the volume output, and the concentrations and outputs of bicarbonate, trypsin, and amylase. The concentration and output of lipase was unaffected.

EFFECT OF HYDROCORTISONE ON RESPONSE TO A CONTINUOUS INFUSION OF SECRETIN Five experiments were made on three dogs.

In four an increase in the volume output appeared within five minutes and persisted for up to one hour. The mean increase was approximately 50% above the maximum obtained before the injection of hydrocortisone. The output of bicarbonate and amylase was increased in four experiments and the concentrations in three. In the two experiments in which trypsin was estimated the concentration and output were unaffected. Lipase was estimated in only one experiment and the output rose substantially after the injection of hydrocortisone (Fig. 8).

EFFECT OF HYDROCORTISONE ON RESPONSE TO A CONTINUOUS INFUSION OF PANCREOZYMIN Four experiments were made on three dogs.

The results were more variable than those obtained on the response to secretin.

The volume output and the concentrations and outputs of bicarbonate, amylase, and trypsin were all increased in two experiments, reduced in one, and unchanged in the other.

An experiment is illustrated in which an increased response followed the injection of hydrocortisone (Fig. 9).

EFFECT OF PRIOR INJECTION OF HYDROCORTISONE UPON THE RESPONSE TO SINGLE INJECTIONS OF THE PANCREATIC HORMONES Hydrocortisone, 100 mg., was injected intravenously 10 to 15 minutes before

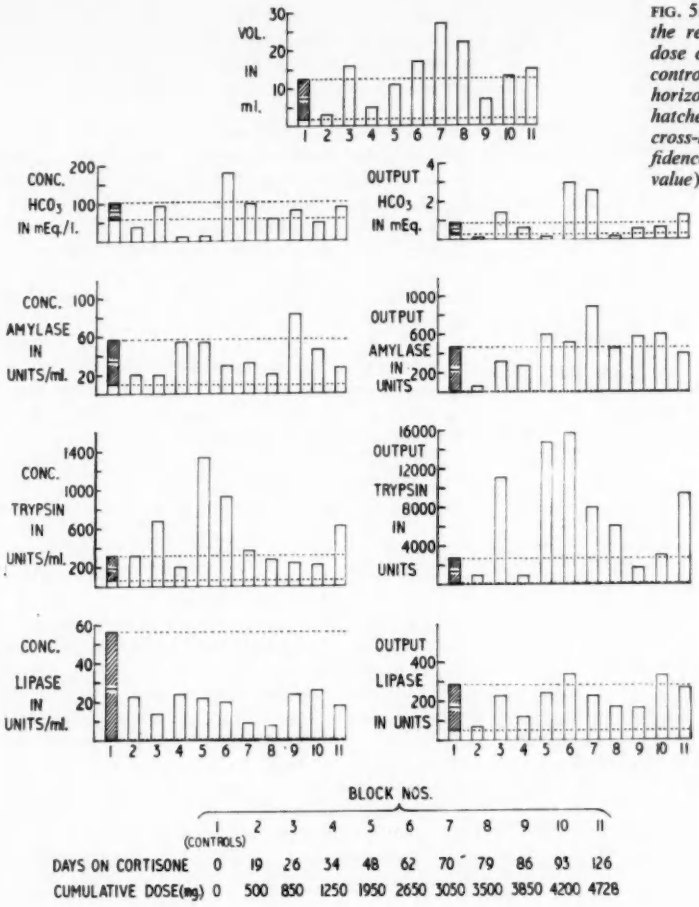
VOL. IN ml.
CONC. HCO3 IN mEq./l.
OUTPUT HCO3 IN mEq.
CONC. AMYLASE IN UNITS/ml.
OUTPUT AMYLASE IN UNITS
CONC. TRYPSIN IN UNITS/ml.
OUTPUT TRYPSIN IN UNITS
CONC. LIPASE IN UNITS/ml.
OUTPUT LIPASE IN UNITS
BLOCK NOS.
1 (CONTROLS) 2 3 4 5 6 7 8 9 10 11
DAYS ON CORTISONE 0 19 26 34 48 62 70 79 86 93 126
CUMULATIVE DOSE(mg) 0 500 850 1250 1950 2650 3050 3500 3850 4200 4728

FIG. 5. *Dog F. The effect of cortisone on the response to a standard intravenous dose of pancreozymin. The mean of the control values is represented by the horizontal line interrupting the cross-hatched first block, and the extent of the cross-hatching indicates the 1 in 40 confidence limits (2 or 2·5% probability value).*

injecting secretin or pancreozymin. Two experiments were made with each hormone in two dogs, and in all four there appeared a substantial increase in the concentration and output of bicarbonate and amylase (Figs. 10 and 11). The other enzymes were not determined in this group of experiments.

The effect of injecting hydrocortisone 30 minutes before secretin and 60 minutes before pancreozymin was tried. Seven control experiments determined the standard response. Two experiments were made with each hormone after the administration of the steroid. In none of the four experiments was any effect noted upon volume, concentration, or output of bicarbonate, amylase, lipase, and trypsin.

In most of the experiments in which an increased volume response was obtained there also appeared an increase in the concentrations of the bicarbonate and enzymes. Thus the augmentation of output after hydrocortisone is unlikely to be the result of an increased 'washing-out' effect of enzymes from the duct system.

DISCUSSION

The results clearly indicate that the pancreatic response, both of electrolytes and enzymes, to stimulation by meals, secretin, and pancreozymin can be influenced by corticotrophin and steroids.

The depression of pancreatic secretion after corticotrophin was noted in a single dog but the result confirms the observations of Pfeffer and Hinton and Dreiling *et al.* previously quoted. In

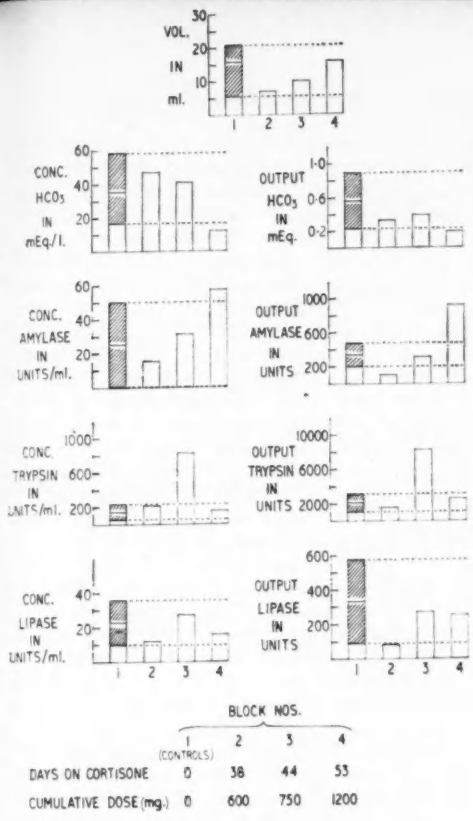
VOL. IN ml.
CONC. HCO3 IN mEq./l.
OUTPUT HCO3 IN mEq.
CONC. AMYLASE IN UNITS/ml.
OUTPUT AMYLASE IN UNITS
CONC. TRYPSIN IN UNITS/ml.
OUTPUT TRYPSIN IN UNITS
CONC. LIPASE IN UNITS/ml.
OUTPUT LIPASE IN UNITS
BLOCK NOS.
1 (CONTROLS) 2 3 4
DAYS ON CORTISONE 0 38 44 53
CUMULATIVE DOSE (mg.) 0 600 750 1200

FIG. 6

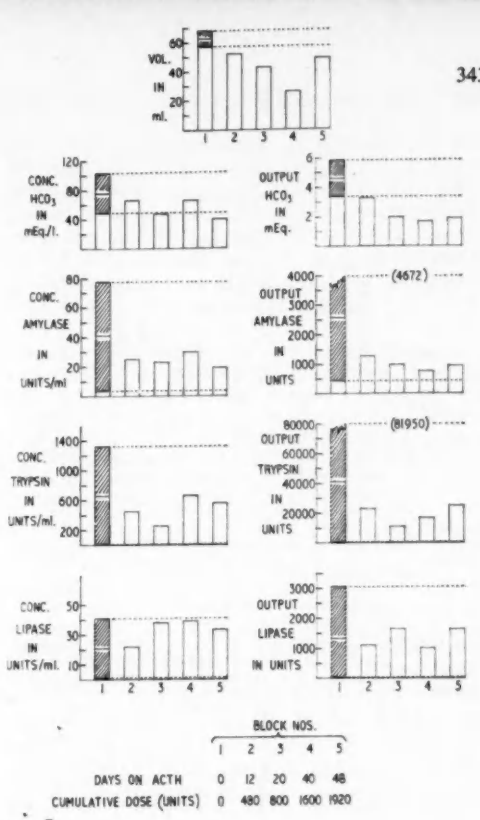
VOL. IN ml.
CONC. HCO3 IN mEq./l.
OUTPUT HCO3 IN mEq.
CONC. AMYLASE IN UNITS/ml
OUTPUT AMYLASE IN UNITS
(4672)
CONC. TRYPSIN IN UNITS/ml.
OUTPUT TRYPSIN IN UNITS
(81950)
CONC. LIPASE IN UNITS/ml.
OUTPUT LIPASE IN UNITS
BLOCK NOS.
1 2 3 4 5
DAYS ON ACTH 0 12 20 40 48
CUMULATIVE DOSE (UNITS) 0 480 800 1600 1920

FIG. 7

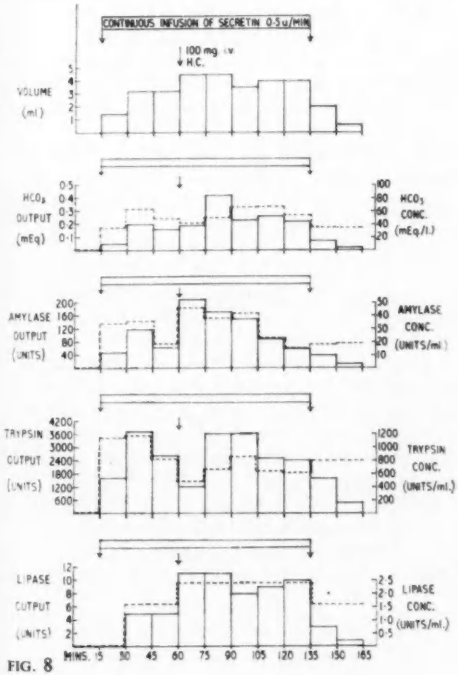
CONTINUOUS INFUSION OF SECRETIN 0·5 u/MIN
100 mg. i.v. H.C.
VOLUME (ml.)
HCO3 OUTPUT (mEq.)
HCO3 CONC. (mEq./l.)
AMYLASE OUTPUT (UNITS)
AMYLASE CONC. (UNITS/ml.)
TRYPSIN OUTPUT (UNITS)
TRYPSIN CONC. (UNITS/ml.)
LIPASE OUTPUT (UNITS)
LIPASE CONC. (UNITS/ml.)
MINS. 15 30 45 60 75 90 105 120 135 150 165

FIG. 8

FIG. 6. *Dog E. The effect of cortisone on the response to a standard intravenous dose of pancreozymin. The mean of the control values is represented by the horizontal line interrupting the cross-hatched first block, and the extent of the cross-hatching indicates the 1 in 40 confidence limits (2 or 2·5% probability value).*

FIG. 7. *The effect of corticotrophin on the response to a standard meal. The mean of the control values is represented by the horizontal line interrupting the cross-hatched first block, and the extent of the cross-hatching indicates the 1 in 40 confidence limits (2 or 2·5% probability value).*

FIG. 8. *The effect of hydrocortisone (H.C.) on the pancreatic response to a continuous infusion of secretin. A typical experiment (9 March 1959).*

——————— = output

– – – – – – – – – = concentration

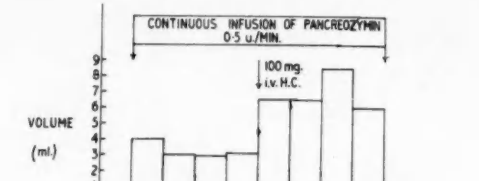

FIG. 9

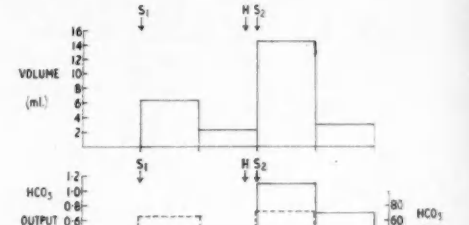

FIG. 10

FIG. 9. *The effect of hydrocortisone on the pancreatic response to a continuous infusion of pancreozymin (18 March 1959).*

———— = *output*
– – – – – – – – – – = *concentration*

FIG. 10. *The effect of hydrocortisone injected 10 minutes beforehand on the pancreatic response to a single injection of secretin (29 April 1960).*

S1. 60 units secretin in 1 ml. distilled water intravenously.
S2. 60 units secretin in 1 ml. distilled water intravenously.
H. 100 mg. hydrocortisone hemisuccinate sodium in 1 ml. distilled water intravenously.

———— = *output*
– – – – – – – – – – = *concentration*

FIG. 11. *The effect of hydrocortisone injected 15 minutes beforehand on the pancreatic response to a single injection of pancreozymin (4 May 1960).*

P1. 100 units pancreozymin in 3 ml. distilled water intravenously.
P2. 100 units pancreozymin in 3 ml. distilled water intravenously.
H. 100 mg. hydrocortisone hemisuccinate sodium in 3 ml. distilled water intravenously.

———— = *output*
– – – – – – – – – – = *concentration*

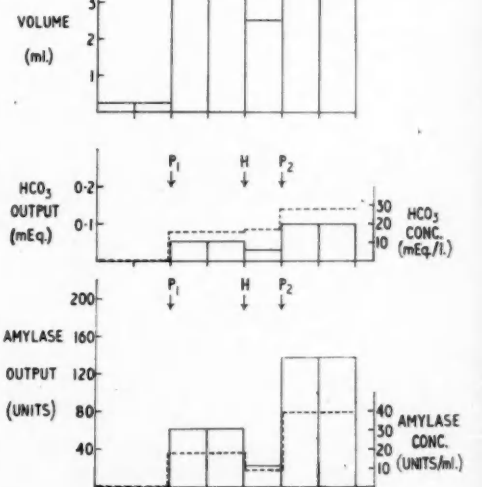

FIG. 11

the Edinburgh Gastrointestinal Unit we have noted that profound depression of gastric secretion may accompany the prolonged administration of corticotrophin to patients (Crean, 1961) although the effect is unpredictable in the individual subject. Hirschowitz (1956) reported similar depression of gastric secretion with dramatic clinical sequelae in volunteer human subjects infused with corticotrophin. It is only possible to speculate on the significance and mechanism of the depressed secretory response of exocrine glands after the administration of corticotrophin, as the available preparations are crude with variable potency and, possibly, of variable chemical constituency. On the one hand, corticotrophin has been shown capable of producing acinar ectasia and fat necrosis (Carone and Liebow, 1957) and, on the other, hypophysectomy may result in atrophy of the pancreas with repair on the subsequent administration of corticotrophin (Patterson, Dingman, Shwachman, and Thorn, 1954; Kinash and Haist, 1954).

There were striking differences in the effect of cortisone and hydrocortisone upon bicarbonate and the enzymes. In acute experiments the concentration and output of bicarbonate were usually raised whereas prolonged administration of hormones appeared to depress the bicarbonate response, as noted by previous workers. But in both acute and chronic experiments the effect of cortisone and hydrocortisone was essentially to increase the concentrations and outputs of the enzymes although the relative resistance of the secretion of lipase to these influences is notable. The difference in the bicarbonate responses in acute and chronic experiments is as yet inexplicable, especially as structural hyperplasia in the pancreas of experimental animals given cortisone has been found to affect both the epithelium of the ducts (Bencosme and Lazarus, 1956) and acinar cells (Sesso and Oliverio, 1959). At whatever site the electrolytes and enzymes of pancreatic secretion are respectively produced, both may be affected.

It has been postulated (Green, 1941) that hormones act by participating in, or specifically affecting, enzyme systems. It may be that the enzyme systems involved in the intracellular production of exocrine secretions are similar in the different glands of the alimentary tract. Thus studies with thiadiazole compounds have suggested that carbonic anhydrase plays a part in both gastric and pancreatic secretion (Davies, 1951; Birnbaum and Hollander, 1953). The immediate effect of steroids upon the intracellular enzymes could result in augmenting the secretory output, while the cumulative effects of prolonged administration may be exerted through the structural hyperplasia previously reported. It was found that the action of a single injection appeared within five minutes and was never prolonged beyond one hour. Furthermore, the effect was so transient that if it was given 30 minutes or more before the injection of hormones which stimulate the pancreas no effect could be discerned. I have also noted similar characteristics in the effect of steroids on gastric secretion in dogs.

I wish to thank Mr. W. Forshall, of the Biochemistry Laboratory of the Edinburgh Northern Hospitals Group, for most of the biochemical estimations, and also the Director, Dr. L. Tompsett; Dr. E. Morton, of Boots Pure Drug Co. Ltd., for supplying part of the secretin and pancreozymin; Mr. D. Wright, of the Department of Medicine, for the diagrams; and Mr. S. A. Sklaroff for help with statistics.

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REFERENCES

Bencosme, S. A., and Lazarus, S. S. (1956). The pancreas of cortisone-treated rabbits. *A.M.A. Arch. Path.*, **62**, 285-295.

Birnbaum, D., and Hollander, F. (1953). Inhibition of pancreatic secretion by the carbonic anhydrase inhibitor, Diamox. *Amer. J. Physiol.*, **174**, 191-195.

Carone, F. A., and Liebow, A. A. (1957). Acute pancreatic lesions in patients treated with ACTH and adrenal corticoids. *New Engl. J. Med.*, **257**, 690-697.

Crean, G. P. (1961). The influence of corticotrophin and corticosteroids on human gastric secretion. *Proc. internat. Congr. Gastroenterol.*, 1960, Series 31, p. 373, Excerpta Medica Foundation, Amsterdam.

Davies, R. E. (1951). The mechanism of hydrochloric acid production by the stomach. *Biol. Rev.*, **26**, 87-120.

Dreiling, D. A., and Janowitz, H. D. (1959). The secretion of electrolytes by the human pancreas. The effect of Diamox, ACTH, and disease. *Amer. J. dig. Dis.*, n.s. **4**, 137-144.

——, ——, and Rolbin, H. (1958). Effect of ACTH and adrenocortical steroids on external pancreatic secretion in man. *New Engl. J. Med.*, **258**, 603-605.

Gowenlock, A. H. (1953). The estimation of tryptic activity in duodenal contents. *Biochem. J.*, **53**, 274-277.

Green, D. E. (1941). Enzymes and trace substances. *Advanc. Enzymol.*, **1**, 177-198.

Hayano, M., and Dorfman, R. I. (1951). Studies on the inhibition of various enzymes by steroids. *Ann. N.Y. Acad. Sci.*, **54**, 608-618.

Hirschowitz, B. (1956). Proceedings of the Gastroenterology Research Group. *Gastroenterology*, **31**, 419-421.

Kinash, B., and Haist, R. E. (1954). Effect of ACTH and of cortisone on islets of Langerhans and the pancreas in intact and hypophysectomized rats. *Amer. J. Physiol.*, **178**, 441-444.

Lagerlöf, H. O. (1942). Pancreatic function and pancreatic disease studied by means of secretin. *Acta med. scand.*, Suppl. 128.

Patterson, P. R., Dingman, J. F., Shwachman, H., and Thorn, G. W. (1954). Choleretic action of cortisone. *New Engl. J. Med.*, **251**, 502-508.

Pfeffer, R. B., and Hinton, J. W. (1956). Some relationships between adrenal medullary and cortical substances and exocrine functions of the pancreas in man. *Gastroenterology*, **31**, 746-757.

Sammons, H. G., Frazer, A. C., and Thompson, M. (1956). The estimation of true lipase in small intestinal contents. *J. clin. Path.*, **9**, 379-380.

Sesso, A., and Oliverio, L. G. (1959). The anabolic action of cortisone on the exocrine pancreas of the suckling rat. *Acta physiol. lat.-amer.*, **9**, 13-23.

Umbreit, W. W. (1951). The influence of adrenalectomy and cortisone treatment on enzymatic reactions in rat tissues. *Ann. N.Y. Acad. Sci.*, **54**, 569-574.

Gut, 1961, **2**, 346

Light- and electron-microscope studies of the effects of 4-aminopteroylglutamic acid (aminopterin) on the mucous membrane of the small intestine of the rat

A. WYNN WILLIAMS

From the Department of Pathology, University of Edinburgh

SYNOPSIS Aminopterin, a cytotoxic agent, causes mitotic arrest by interfering with the catabolism of ribonucleoprotein. The effects on the small intestine are demonstrated. The resulting atrophy, with changes in the microvilli, is associated with severely impaired fat absorption.

The essential cause of the abnormal light microscopic appearances of human jejunal mucous membrane in malabsorption disease is unknown and it has not been decided whether the epithelial changes are the primary cause of malabsorption and nutritional deficiency or whether they are secondary phenomena. So far, experimental attempts to produce strictly comparable chronic intestinal abnormalities in animals have failed. However, gross atrophy of jejunal mucosa was observed in mice (Dustin, 1949; Jacobson, 1951) and in rats (Woll and Oleson, 1951; Vitale, Zamcheck, DiGiorgio, and Hegsted, 1954) given 4-aminopteroylglutamic acid (aminopterin), and the changes deserve special attention not only because human malabsorption may conceivably be dependent on a similar derangement of metabolism but also because cytotoxic drugs are being increasingly used in the treatment of cancer. In this paper a brief account confirming the main findings of these investigators is augmented by a description of electron-microscopic appearances. It is suggested that the latter may be usefully compared or contrasted with ultramicroscopic findings in jejunal biopsies from cases of human malabsorption.

MATERIALS AND METHODS

Sixty adult Wistar rats, kept on a normal diet and allowed as much water as they wished, were injected subcutaneously each day with aminopterin dissolved in saline. The dose was 300 μg. per kg. body weight and groups of six animals were killed daily by a blow on the head. The abdomen was opened and portions of stomach, jejunum, ileum, and large intestine were taken for light and electron microscopy. Blocks for light microscopy were fixed in 10% formol-saline and paraffin sections were stained by haematoxylin and eosin. Tissue for electron microscopy was cut into blocks of approximately 1 c.mm. with a razor blade and placed in Dalton's osmium tetroxide fixative for one hour, dehydrated in graded concentrations of ethanol and embedded in methacrylate (9 parts of butylmethacrylate to 1 part of methylmethacrylate). Sections were cut on a Servall Porter-Blum microtome, using a glass knife. Thicker sections were examined by phase microscopy. Ultrathin sections were examined in a Metropolitan Vickers EM-6 electron microscope. Portions of normal intestine from four control animals, injected with saline only, were also studied. In four of the aminopterin-treated animals killed on the seventh day of the experiment, as well as in two additional control animals, 10 ml. corn oil (Mazola) was administered by stomach tube one hour before killing the animals. Frozen sections of jejunum were stained with Sudan III while portions of jejunal mucosa were examined in the electron microscope.

RESULTS

Four to seven days after starting injections of aminopterin the rats developed diarrhoea. At necropsy the whole of the intestine contained brown watery fluid. There were, however, no inflammatory signs and no haemorrhages. The wall of the small intestine was pale and thinner than normal. Microscopically, there was progressive degeneration and atrophy of the villi, notably in the duodenum and jejunum but also in the ileum. Fig. 1 illustrates the appearance of normal jejunal mucosa in the rat. Five days after the administration of aminopterin, degenerative changes are obvious in the jejunal mucosa (Figs. 2 and 3) and the villi

become stunted and broadened. Some of the epithelial cells show marked hydropic degeneration. Many of the crypts become dilated and lined by thin, atrophic epithelial cells, some showing mitotic arrest. Small numbers of polymorphonuclear leucocytes are seen within the crypts and in the adjacent stroma. The degenerative changes are progressive (Fig. 4) and involve also the ileum (Fig. 5).

No erosions or ulcers were seen at any stage. The stomach appeared normal.

One hour after the administration of corn oil by stomach tube to normal rats the jejunum appears thick and succulent and contains milky fluid in the lumen, and prominent lymphatics are seen in the adjacent mesentery. Moreover, in frozen sections stained with Sudan III fat droplets are numerous within the cytoplasm of the epithelial cells covering the villi. In marked contrast, the jejunum of aminopterin-injected, oil-fed animals is thin, contracted, and pale. The oil remains unabsorbed in the lumen and the mesenteric lymphatics are not seen, while in frozen sections very few cytoplasmic fat droplets are observed and these are confined to a few epithelial cells at the tips of the villi.

In the normal rat, the small intestinal villi are covered by a 'brush border' which is visible in the light microscope as a hazy thickening, or, under the best circumstances, as a striated edge on the epithelial cells. In the electron microscope, the microvilli are seen as a palisade of closely set structures averaging $1{\cdot}3\mu$ in length and $0{\cdot}08\mu$ in width. Their regular arrangement is a striking feature (Fig. 6). It has been calculated that in human jejunum there are 1,800 microvilli per cell and 1×10^9 microvilli per square millimetre of a mucosal surface. A similar figure was obtained in the rat (Ashworth, Chears, Sanders, and Pearce, 1961). These structures are disposed in a manner which facilitates the formation of pinocytotic vesicles in the subjacent cytoplasm by infolding the cell membrane between the microvilli, when the vesicle is pinched off from the membrane. The cytoplasm within the microvilli and immediately beneath them is devoid of mitochondria but there is a dense, well-defined osmophilic band in this zone, corresponding to the 'terminal web' (Sauer, 1937) seen by light microscopy (Fig. 6). Beneath the terminal web, endoplasmic reticulum and mitochondria are dispersed through the upper apical cytoplasm of the cells.

In rats treated with aminopterin for several days the microvilli are markedly abnormal, being very few and extremely short. The diameter remains normal or is slightly increased. Moreover, the terminal web disappears and the mitochondria appear diminished in size. These features are illustrated in Fig. 7.

One hour after the administration of corn oil by stomach tube to normal rats, droplets of characteristic shape are seen within the cytoplasm of the villous epithelium of the jejunum by electron microscopy (Fig. 8). These are numerous round or oval jet black masses 300 Å to 2μ in diameter. Similar oil droplets are absent (Fig. 9) or very scanty in the epithelial cells of aminopterin-treated rats except in occasional cells near the tip of the villus where the brush border was seen to be better preserved (Fig. 10).

DISCUSSION

The cause and significance of the mucosal abnormalities in the small intestine of patients suffering from malabsorption is largely unknown but it is of interest that the most comparable lesions so far produced in the experimental animal are those which follow the administration of antimetabolic substances such as aminopterin. This chemical is an antagonist of folinic acid (leuconostoc citrovorum factor) rather than folic acid and it interferes with the catabolism of ribonucleoprotein (Jacobson, 1951, 1954). The latter investigator demonstrated that aminopterin arrests dividing thick fibroblasts and osteoblasts, also leukaemic cells in metaphase, so that the nuclei are unable to proceed into anaphase. The epithelial cells covering intestinal villi have a life span of a few days. They originate in the crypts of Lieberkühn, where normally there is intense mitotic activity, and they gradually migrate along the surface of the villus until they are shed near or at the tip. Aminopterin produces mitotic arrest in the crypts and, consequently, fewer epithelial cells are available to cover the villus. The cells already present are often larger than normal and cover a larger surface of the villus. A similar sequence of mitotic arrest and epithelial degeneration is seen after abdominal irradiation by *x* rays (Friedman, 1945). It might be assumed that such cellular expansion would account for the changes in the brush border in aminopterin-treated rats but in fact distension of the bowel of rats does not lead to such gross abnormalities in the brush border (Muir, 1961), and it seems that the cause must reside within the cell. The function of microvilli has not been definitely determined but there is evidence that they are concerned in fat absorption. Palay and Karlin (1956), also Thomas and O'Neal (1960), have suggested that fat may be absorbed in particulate form from the lumen of the intestine. Whether this view is correct or not, the observation reported here that fat is absorbed in aminopterin-

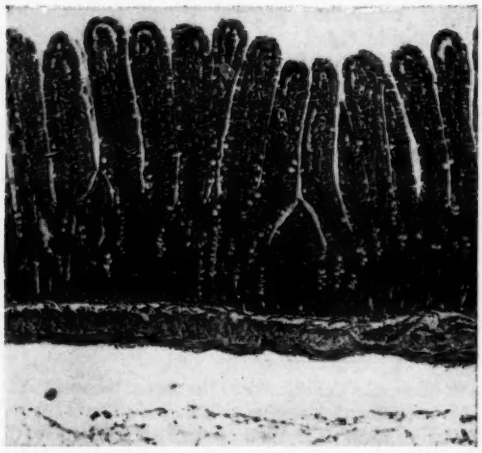

FIG. 1. *Normal rat jejunum. Haematoxylin and eosin,* × 60.

FIG. 2. *Jejunum of rat given aminopterin for five days. Haematoxylin and eosin,* × 60.

FIG. 5. *Degenerate mucosa in ileum of rat given aminopterin for seven days. Haematoxylin and eosin,* × 60.

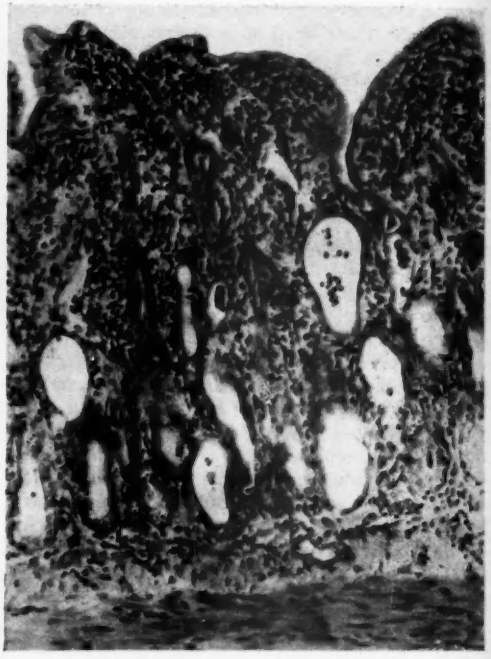

FIG. 3. *Jejunum of rat given aminopterin for five days. Haematoxylin and eosin,* × 130.

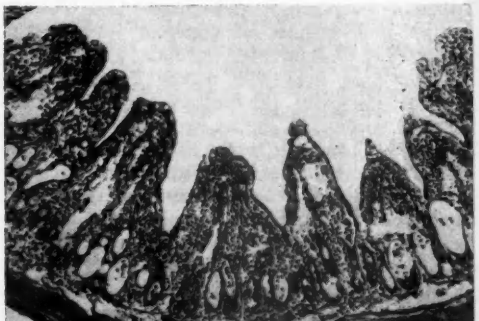

FIG. 4. *Advanced degenerative change in jejunum of rat given aminopterin for seven days. Haematoxylin and eosin,* × 60.

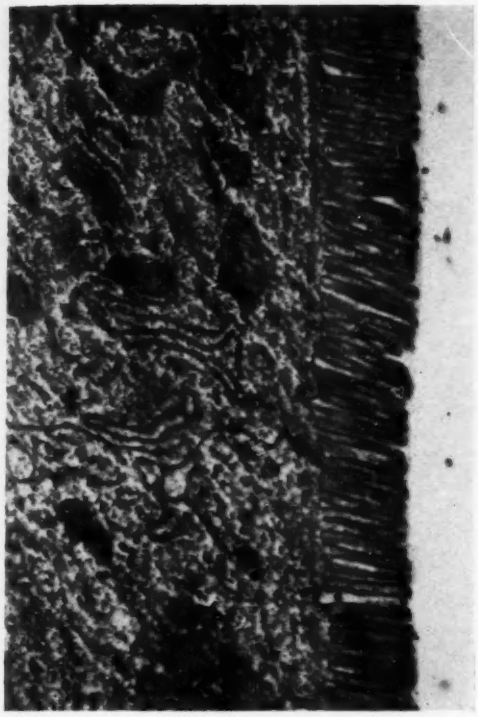

FIG. 6

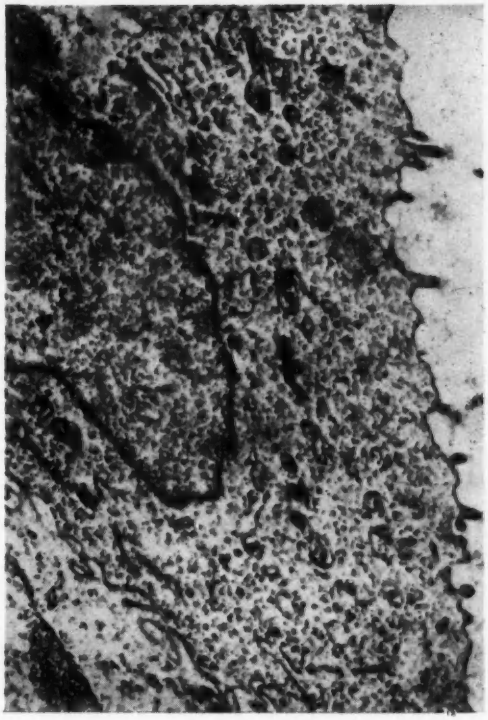

FIG. 7

FIG. 6. *Brush border in normal rat jejunum. Portions of two cells are seen, with prominent mitochondria. The terminal web is the dark horizontal line beneath the microvilli.* × *20,000.*

FIG. 7. *Abnormal brush border in jejunum of rat given aminopterin for seven days.* × *20,000.*

treated rats only in those few intestinal cells in which the brush border was relatively normally preserved supports the concept that the latter is necessary for fat transportation. It is to be expected, therefore, that any reduction in the villous and, more particularly, the microvillous surface of the small bowel must drastically reduce its absorptive capacity. Blunted villi may reduce the surface area to 20% of normal (Butterworth and Perez-Santiago, 1958), while it has been estimated that the microvilli may account for 95% of the absorptive area of the small intestine (Hartman, 1959). In primary malabsorption disease in man the villi are often absent or abnormal (Shiner, 1959; Shiner and Doniach, 1960; Fone, Cooke, Meynell, Brewer, Harris, and Cox, 1960) but in some cases light microscopic findings are normal (Girdwood, Delamore, and Wynn Williams, 1961). The latter investigators studied jejunal biopsies from 24 patients with malabsorptive disease of the idiopathic steatorrhoea type and found no obvious correlation between the type of mucosal appearance and the results of laboratory tests of absorption. Moreover, there was nothing in their results to justify the view that primary malabsorptive disease with absent villi had a different aetiology and symptoms from primary malabsorptive disease with normal villi. Electron-microscopic studies of human biopsy material may, however, reveal significant abnormalities in the brush border, as has been suggested in one brief report by Ashworth *et al.* (1961).

I wish to acknowledge the encouragement of Professor G. L. Montgomery and Dr. R. H. Girdwood in this

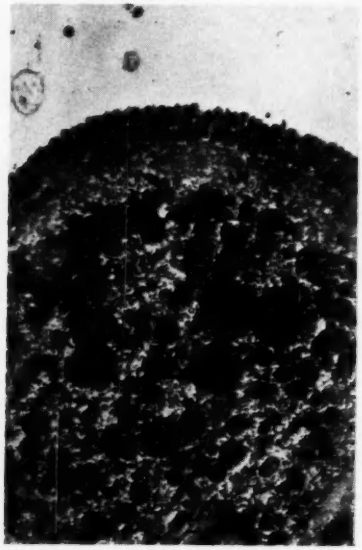

FIG. 8. *Part of an epithelial cell covering a jejunal villus in a normal rat, one hour after giving corn oil by mouth.* × *8,000.*

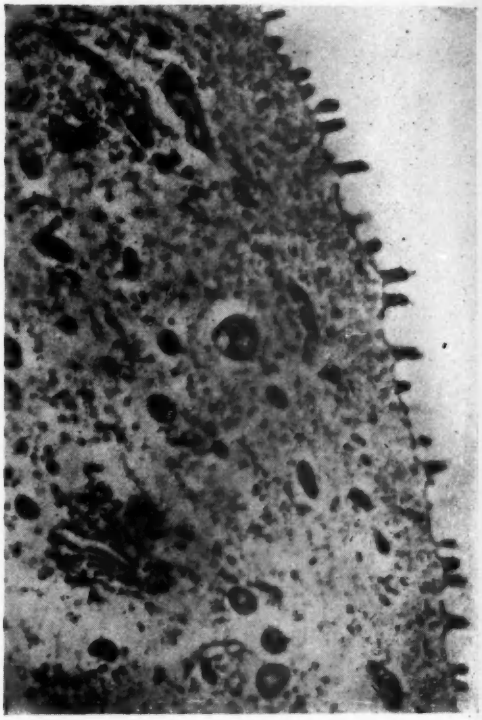

FIG. 9. *Part of an epithelial cell covering a jejunal villus in a rat given aminopterin for seven days and corn oil by mouth one hour before death. There are no lipid droplets in the cytoplasm.* × *20,000.*

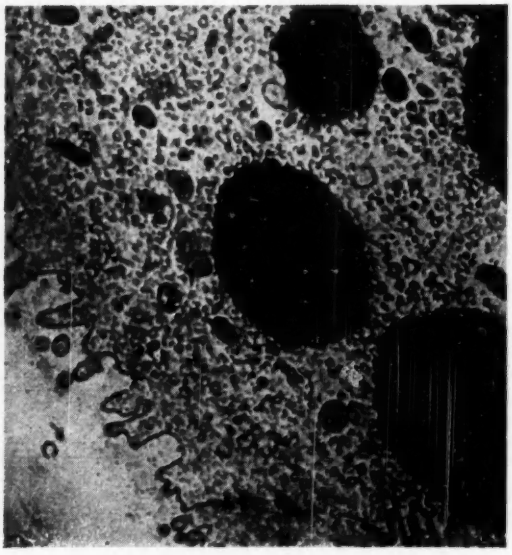

FIG. 10. *Part of another villous epithelial cell from the same animal as in Fig. 9. Here the brush border is partly preserved and a few lipid droplets are present in the cytoplasm.*

study, also the unfailing technical assistance of Dr. Mary MacDonald, Mr. T. C. Dodds, and Mr. George Wilson. I am also most grateful to the Advisory Committee for Medical Research and the Secretary of State for Scotland for generous financial assistance in this work.

REFERENCES

Ashworth, C. T., Chears, W. C., Sanders, E., and Pearce, M. B. (1961). Nontropical sprue. Fine structure of the intestinal epithelial lesion. *Arch. Path.* (*Chicago*), **71**, 25.

Butterworth, C. E., Jr., and Perez-Santiago, E. (1958). Jejunal biopsies in sprue. *Ann. intern. Med.*, **48**, 8.

Dustin, P., Jr. (1949). Action de deux antagonistes de l'acide folique sur les mitoses intestinales de la souris. *C.R. Soc. Biol.* (*Paris*), **143**, 1609.

Fone, D. J., Cooke, W. T., Meynell, M. J., Brewer, D. B., Harris, E. L., and Cox, E. V. (1960). Jejunal biopsy in adult coeliac disease and allied disorders. *Lancet*, **1**, 933.

Friedman, N. B. (1945). Cellular dynamics in the intestinal mucosa: The effect of irradiation on epithelial maturation and migration. *J. exp. Med.*, **81**, 553.

Girdwood, R. H., Delamore, I. W., and Williams, A. Wynn (1961). Jejunal biopsy in malabsorptive disorders of the adult. *Brit. med. J.*, **1**, 319.

Hartman, R. S. (1959). Quoted by Gardner, F. H., and Strauss, E. W., in *Advanc. intern. Med.*, **10**, 137 (1960).

Jacobson, W. (1951). The Biology of Cell Division. In *Conference on Problems of Ageing: Trans. 13th Conference, 1951*, p. 155. Ed. N. W. Shock. Josiah Macy, Jr. Foundation, New York.

——. (1954). The Mode of Action of Folic Acid Antagonists and the Function of the Leuconostoc Citrovorum Factor. In *Ciba Foundation Symposium on Chemistry and Biology of Pteridines*, p. 329. Churchill, London.

Muir, A. R. (1961). Personal communication.

Palay, S. L., and Karlin, L. (1956). Absorption of fat by jejunal epithelium in the rat. *Anat. Rec.*, **124**, 343.

Sauer, F. C. (1937). Some factors in the morphogenesis of vertebrate embryonic epithelia. *J. Morph.*, **61**, 563.

Shiner, M. (1959). Small intestinal biopsy: Diagnostic and research value. *Proc. roy. Soc. Med.*, **52**, 10.

——, and Doniach, I. (1960). Histopathologic studies in steatorrhoea. *Gastroenterology*, **38**, 419.

Thomas, W. A., and O'Neal, R. M. (1960). Electron microscopy studies of butter and corn oil in jejunal mucosa. *A.M.A. Arch. Path.*, **69**, 121.

Vitale, J. J., Zamcheck, N., DiGiorgio, J., and Hegsted, D. M. (1954). Effects of aminopterin administration on the respiration and morphology of the gastrointestinal mucosa of rats. *J. Lab. clin. Med.*, **43**, 583.

Woll, E., and Oleson, J. J. (1951). The effects of a folic acid antagonist (aminopterin) on albino rats: A study in the pathogenesis of sprue. *Brit. J. exp. Path.*, **32**, 458.

Gut, 1961, **2**, 352

Secretion of blood group substances in duodenal, gastric and stomal ulcer, gastric carcinoma, and diabetes mellitus

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SYNOPSIS The ABO blood group and ABH secretor status have been determined for 368 patients with a duodenal ulcer, 202 patients with a gastric ulcer, 83 patients with a stomal ulcer, 105 patients with gastric cancer, 102 patients with diabetes mellitus, and a control group of 610 subjects consisting of healthy persons and of patients with other conditions attending the same hospital.

The results have been analysed in conjunction with those reported in two other large series. For duodenal ulcer the results show that the relative incidence among non-secretors compared with secretors is 1·80 to 1, with 95% confidence limits of 1·55 to 1 and 2·03 to 1. For gastric ulcer the relative incidence is 1·42 to 1, with 95% confidence limits of 1·16 to 1 and 1·74 to 1. Comparison of the results for the two types of peptic ulcer shows that they are significantly different from one another.

For both types of ulcer, the estimated risk among non-secretors was similar for blood group O and for the other blood groups and the results suggest that the specific risks associated with blood group O and with non-secretion multiply one another. It is concluded that the mechanisms by which blood group O and non-secretion affect the risk of developing a gastric or duodenal ulcer are related to one another; but that they do not depend on the presence of the blood group substances in the secretions.

The proportion of non-secretors among patients with a stomal ulcer (49%) was higher than among patients with a duodenal ulcer (37%) and the order of the relationship between non-secretion and the three types of peptic ulcer (stomal, duodenal, and gastric) was the same as that for blood group O.

Other data suggest that there may be a slight increase in the risk of gastric cancer among non-secretors, but that the occurrence of diabetes mellitus is independent of ABH secretion.

Interest in the association between gastrointestinal disease and the ABO blood group substances was enhanced by the discovery that there was also an association between duodenal ulcer and failure to secrete the substances in the mucous secretions (Clarke, Edwards, Haddock, Howel-Evans, McConnell, and Sheppard, 1956; Clarke, Evans, McConnell, and Sheppard, 1959). Not everyone is capable of secreting these substances; but, among those who are, the substances form a major part of the soluble mucus. It seemed reasonable, therefore, to suppose that the association between particular diseases and the different blood groups might be due to a difference in the quality of the protective power of the different types of mucus. One finding weighed against this explanation. According to Clarke and his colleagues the association between duodenal ulcer and group O was no stronger among secretors than among non-secretors, whereas if the effect of the group substances was exerted locally in the lumen of the gut, it would have been expected that the association would have been limited to secretors.

Our inquiry was undertaken (1) to see if the observations on duodenal ulcer could be confirmed, and (2) to investigate the incidence of non-secretors in some other diseases which were known to be associated with one or other of the ABO blood groups.

METHOD

Secretion of the ABO blood group substances was tested by seeing whether the subject's saliva would prevent the agglutination of group O red cells by an extract of the seeds of *Ulex europaeus*. This extract reacts with the H antigen common to all the ABO blood groups and has been found to react with the saliva of all secretors, irrespective of the blood group to which they belong (Boyd and Shapleigh, 1954; Clarke *et al.*, 1956). The adequacy of the method was confirmed in a few subjects belonging to groups A, B, and AB by the use of anti-A or anti-B sera and, in selected subjects belonging to groups O and A, by testing for the presence of Lewis antigens in the blood. Apart from a few subjects who were negative for both the Lewis antigens, all non-secretors were positive for Lewis a (Le a+) and all secretors positive for Lewis b (Le b+).

The patients attended the Central Middlesex Hospital. No attempt was made to obtain a consecutive series but care was taken to ensure that the choice was not influenced by knowledge of the ABO blood group. Patients were selected because they were known to be suffering from a peptic ulcer, gastric cancer, diabetes mellitus, or ulcerative colitis. Other persons examined consisted of (1) patients admitted to the general medical and surgical wards of the hospital who were not suffering from any of the above conditions, and (2) healthy subjects, *i.e.*, blood donors, members of the hospital staff, medical colleagues, personal friends, and the spouses of patients.

Patients were excluded if they suffered from a condition suggestive of a peptic ulcer, without the presence of an ulcer having been established, or if they were known to have had both gastric and duodenal ulcers. Africans and West Indians were excluded irrespective of the condition from which they suffered.

Data on patients with ulcerative colitis are omitted from the present report, as the number of patients studied (44) is too small for separate analysis.

RESULTS

Data obtained from 385 healthy subjects and 225 patients suffering from conditions other than those under special study are shown in Table I. The results are closely similar in the two groups and they have, therefore, been combined to provide a single control series. Table II shows the distribution of the ABO blood groups in the combined series as compared with that reported by Discombe and Meyer (1952) for 10,000 women attending ante-natal clinics in the area of the Central Middlesex Hospital. The two sets of results are almost identical. Table III shows the proportion of non-secretors in the control series compared with that reported by Glynn, Glynn, and

TABLE I

NUMBER OF SECRETORS AND NON-SECRETORS AMONG CONTROL SUBJECTS BY SEX AND BLOOD GROUP

Category of Subjects	*Sex*	*Number of Persons in Blood Group*								*Total Number of Subjects*	*Percentage Non-secretors*
		O		*A*		*B*		*AB*			
		Secretors	*Non-secretors*	*Secretors*	*Non-secretors*	*Secretors*	*Non-secretors*	*Secretors*	*Non-secretors*		
Healthy subjects	M	49	11	55	15	16	6	1	0	153	21·1
	F	81	28	81	16	14	5	6	1	232	21·6
	M+F	130	39	136	31	30	11	7	1	385	21·3
Patients suffering from conditions other than those under special study	M	39	8	29	4	7	2	1	1	91	16·5
	F	46	10	44	18	6	4	6	0	134	23·9
	M+F	85	18	73	22	13	6	7	1	225	20·9
All categories	M	88	19	84	19	23	8	2	1	244	19·3
	F	127	38	125	34	20	9	12	1	366	22·4
	M+F	215	57	209	53	43	17	14	2	610	21·1

Difference between proportions of non-secretors in:
Men and women $\chi^2 = 0{\cdot}857$, n = 1, $0{\cdot}30 < P < 0{\cdot}50$
Healthy subjects and hospital patients $\chi^2 = 0{\cdot}014$, n = 1, $0{\cdot}90 < P < 0{\cdot}95$
Groups O, A, and B plus AB $\chi^2 = 0{\cdot}816$, n = 2, $0{\cdot}50 < P < 0{\cdot}70$

TABLE II

DISTRIBUTION OF ABO BLOOD GROUPS IN CONTROL SERIES AND COMPARED WITH THAT IN A PREVIOUS SERIES FROM SAME AREA

Series	*Percentages of Persons in Blood Group*				*Total Number of Subjects*
	O	*A*	*B*	*AB*	
Present control series	44·6	43·0	9·8	2·6	610 (100·0%)
Discombe and Meyer (1952)	45·8	42·2	8·9	3·1	10,000 (100·0%)

TABLE III

NUMBER OF SECRETORS AND NON-SECRETORS AMONG CONTROL SUBJECTS BY SEX AND BLOOD GROUP IN COLLECTED SERIES

Series	Sex	Number of Persons in Blood Group								Total Number of Subjects	Percentage of Non-secretors
		O		A		B		AB			
		Secretors	Non-secretors	Secretors	Non-secretors	Secretors	Non-secretors	Secretors	Non-secretors		
Glynn *et al.* (1959)	M & F	—	—	—	—	—	—	—	—	669	22·9
McConnell (1961a, 1961b)	M	144	46	138	42	46	12	10	2	440	21·2
	F	172	48	116	42	21	12	7	2	421	24·9
Newman *et al.* (1961)	M	250	69	233	75	66	19	20	7	739	23·0
	F	197	47	154	50	41	18	10	5	522	23·0
Present control series	M	88	19	84	19	23	8	2	1	244	19·3
	F	127	38	125	34	20	9	12	1	366	22·4

Difference between proportions of non-secretors in:
Men and women $\chi^2 = 0{\cdot}672$, n = 1, $0{\cdot}3 < P < 0{\cdot}5$
Groups O, A, B, and AB $\chi^2 = 3{\cdot}446$, n = 3, $0{\cdot}3 < P < 0{\cdot}5$

Holborow (1959) for school children in Slough, 15 miles west of the hospital; by McConnell (1961a, and personal communication, 1961b) for healthy persons in Liverpool; and by Newman, Naifeh, Auer, and Buckwalter (1961) for hospital personnel and selected patients in Iowa, U.S.A. The results in all four series are clearly similar. Analysis of the three series which give results separately for men and for women and for each of the ABO blood groups shows that there is no reason to suppose that the proportion of non-secretors varies between the different blood groups, nor that it varies between men and women. It will, however, be seen later that the proportion of non-secretors is usually higher among women than among men in the pathological series and there is reason to believe that there may be a sex difference in the concentrations of the blood group substances in the saliva (Doll and Drane, 1961). Data for the two series have, therefore, been shown separately for each sex throughout the present report, and all comparisons between groups of subjects have been made independently for men and for women.

DUODENAL ULCER The results obtained in patients with a duodenal ulcer are shown in Table IV. Similar data have been published for two other large series and these are included in Table IV for comparison. Since the numbers of patients belonging to the rarer blood groups are small, it has been thought more useful to analyse the results for all these series together, rather than to examine the present results independently. This is justified as it will be shown that the results of the three series are not significantly heterogeneous.

TABLE IV

NUMBER OF SECRETORS AND NON-SECRETORS AMONG DUODENAL ULCER PATIENTS BY SEX AND BLOOD GROUP IN COLLECTED SERIES

Series	Sex	Number of Persons in Blood Group								Total Number of Persons	Percentage of Non-secretors
		O		A		B		AB			
		Secretors	Non-secretors	Secretors	Non-secretors	Secretors	Non-secretors	Secretors	Non-secretors		
McConnell (1961a, 1961b)	M	303	162	184	84	36	24	17	5	815	33·7
	F	67	36	37	25	13	3	4	0	185	34·6
Newman *et al.* (1961)	M	156	62	83	44	22	13	8	3	391	31·2
	F	28	11	19	17	7	2	3	1	88	35·2
Present duodenal ulcer patients	M	100	52	76	40	7	7	5	4	291	35·4
	F	23	17	15	13	5	3	2	0	78	42·3

Mean relative incidence among non-secretors compared with secretors, total data,
χ^2 for the difference from unity = $62{\cdot}229$, n = 1, $P < 0{\cdot}001$
χ^2 for homogeneity in 18 subgroups = $15{\cdot}046$, n = 17, $0{\cdot}3 < P < 0{\cdot}5$
χ^2 for homogeneity among men and women = $0{\cdot}063$, n = 1, $0{\cdot}8 < P < 0{\cdot}9$
χ^2 for homogeneity among blood groups O, A, and B plus AB = $3{\cdot}938$, n = 2, $0{\cdot}1 < P < 0{\cdot}2$
χ^2 for homogeneity among the three series = $3{\cdot}519$, n = 2, $0{\cdot}1 < P < 0{\cdot}2$

Comparison of the data shown in Tables III and IV shows that in each series the proportion of non-secretors is higher among patients with a duodenal ulcer than among the corresponding control subjects. The importance of the difference may be assessed by calculating the relative incidence of duodenal ulcer among non-secretors and secretors. This can be done with the present data, on the assumption that the control subjects are representative in respect of ABO blood group distribution and secretor status of the populations from which the corresponding duodenal ulcer patients were drawn. With Woolf's method (Woolf, 1955; Roberts, 1957) it is possible to calculate a 'mean relative incidence' for several series, taking account of the varying proportions of duodenal ulcer patients and controls in the different series and the possibility that the proportion of non-secretors may be different in different subgroups of the control population.

Since the numbers of women patients in blood groups B and AB are small, the data for these two blood groups have been combined. The relative incidence of duodenal ulcer among non-secretors and secretors has, therefore, been calculated separately for the 18 subgroups given by the three series, three blood groups (O, A, and B plus AB) and two sexes. The mean relative incidence of duodenal ulcer among non-secretors compared with that among secretors is calculated to be 1·80 to 1; that is, the incidence of duodenal ulcer is calculated to be 80% higher among non-secretors than among secretors. The excess is statistically highly significant ($P < 0.001$) and, because of the large number of patients, it can be regarded as accurate within fairly narrow limits. Woolf's method gives fiducial limits for the mean relative incidence of 1·55 to 1 and 2·03 to 1; that is, there is a 95% chance that the true value for the mean relative incidence lies within these limits.

Further analysis shows that the results obtained in the 18 subgroups are consistent with one another and a direct comparison shows no difference between the results[1] obtained for men and women, blood groups O, A, and B plus AB, and the three separate series.

GASTRIC ULCER The results obtained in patients with a gastric ulcer are shown in Table V. As previously, the results of other studies are included for comparison. The data reported by McConnell (1961b) include cases reported previously by Clarke *et al.* (1959) and by McConnell (1961a); but they exclude cases in which both gastric and duodenal ulcers were present and all cases which were selected as a result of knowledge of their blood groups.

Comparison of the data in Tables III and V shows that in each series the proportions of non-secretors are higher among patients with a gastric ulcer than among the corresponding control subjects. From all the data the mean relative incidence of gastric ulcer among non-secretors compared with that among secretors is calculated to be 1·42 to 1; that is, the incidence of gastric ulcer is 42% higher among non-secretors than among secretors. The excess is statistically highly significant ($P < 0.001$) and there is a 95% chance that the true value of the relative incidence lies between the limits of 1·16 and 1·74 to 1. As in the case of duodenal ulcer, the data can be regarded as homogeneous and there is no need to postulate any difference between the relative incidence for men and for women, the three principal

[1]The method of analysis is described in the appendix.

TABLE V

NUMBER OF SECRETORS AND NON-SECRETORS AMONG GASTRIC ULCER PATIENTS BY SEX AND BLOOD GROUP IN COLLECTED SERIES

Series	*Sex*	*Number of Persons in Blood Group*								*Total Number of Patients*	*Percentage of Non-secretors*
		O		*A*		*B*		*AB*			
		Secretors	*Non-secretors*	*Secretors*	*Non-secretors*	*Secretors*	*Non-secretors*	*Secretors*	*Non-secretors*		
McConnell (1961b)	M	50	20	40	15	4	4	0	0	133	29·3
	F	32	16	19	11	16	4	1	0	99	31·3
Newman *et al.* (1961)	M	57	23	35	10	13	2	4	2	146	25·3
	F	18	6	6	3	3	1	1	0	38	26·3
Present gastric ulcer patients	M	31	13	32	13	6	2	1	1	99	29·3
	F	42	18	22	14	4	1	1	1	103	33·0

Mean relative incidence among non-secretors compared with secretors, total data,
χ^2 for the difference from unity = 11·636, n = 1, $P < 0.001$
χ^2 for homogeneity in 18 subgroups = 8·881, n = 17, $0.90 < P < 0.95$
χ^2 for homogeneity among men and women = 2·527, n = 1, $0.1 < P < 0.2$
χ^2 for homogeneity among blood groups O, A, and B plus AB = 1·982, n = 2, $0.1 < P < 0.2$
χ^2 for homogeneity among the three series = 1·570, n = 2, $0.2 < P < 0.3$

blood groups O, A, and B plus AB, or the three separate series. It may be noted, however, that if the three series are considered independently only one provides clear evidence of a significant excess ($P < 0.01$); in another, the excess is of borderline significance ($P = 0.05$) and in the third it is not significant ($P = 0.2 < P < 0.3$).

The increase in the incidence of gastric ulcer among non-secretors (42%) is less than that recorded for duodenal ulcers (80%). The difference is substantial and a direct comparison of the data in Tables IV and V shows that the proportions of non-secretors among patients with the two types of ulcers are significantly different ($\chi^2 = 5.148$, $n = 1$, $P = 0.03$).

STOMAL ULCER The results obtained in 83 patients with one or other form of stomal ulcer are shown in Table VI. The only other published data for stomal ulcers were obtained in part by a different technique (Wallace, Brown, Cook, and Melrose, 1958); the two series are not fully comparable and the contents of Table VI are, therefore, limited to the data obtained in the present series. Comparison of the figures in Tables III, IV, and VI shows that in each sex the proportion of non-secretors is substantially higher among stomal ulcer patients than among either the control subjects or patients with a duodenal ulcer.

The numbers of stomal ulcer patients in some of the groups are small and it is not practicable to analyse the results separately for blood groups A, B, and AB. The data for persons belonging to these blood groups have, therefore, been combined and the mean relative incidence of stomal ulcer has been calculated from the data for men belonging to blood group O, men belonging to other groups, women belonging to blood group O, and women belonging to other groups. From comparison with the control subjects (Table III), it is calculated that the incidence of stomal ulcer is 4·22 times greater among non-secretors than among secretors. This excess is highly significant. The increase in the incidence of stomal ulcers among non-secretors (322%) is substantially greater than that recorded for duodenal ulcers (80%). Since, however, all the stomal ulcer patients were examined at the Central Middlesex Hospital the more correct comparison is with the duodenal ulcers in the present series. In this subgroup the excess incidence among non-secretors was somewhat larger (145%)[2] and the difference between the stomal and the duodenal ulcer patients is only just statistically significant ($P = 0.04$).

From examination of Table VI it appears that the proportion of non-secretors is higher among stomal ulcer patients belonging to blood group A than among patients belonging to group O and that it is higher still among patients belonging to groups B and AB. The numbers of cases are, however, small and a test for homogeneity of the data shows that the variation in the estimates of the relative incidence among non-secretors could easily be due to chance.

CARCINOMA OF STOMACH AND DIABETES MELLITUS Data for 452 cases of carcinoma of the stomach and 420 cases of diabetes mellitus are shown in Table VII. If these are compared with the data for the

[2]The relative incidence of duodenal ulcer among non-secretors, calculated from the results obtained at the Central Middlesex Hospital, was somewhat greater than that obtained from the combined results of all three centres (2·45 to 1 against 1·80 to 1). It has been shown that this difference may well be due to chance (p. 355) and it would have been reasonable to compare the stomal ulcer data with the combined duodenal ulcer data. If this had been done, the difference between the stomal and the duodenal ulcer patients would have been much more marked. There must, however, be some doubt about the complete comparability of the data obtained at the different centres and it seemed safer in this instance to limit the comparison to data obtained at a single centre.

TABLE VI

NUMBER OF SECRETORS AND NON-SECRETORS AMONG PATIENTS WITH A STOMAL ULCER BY SEX AND BLOOD GROUP

Sex	*Number of Persons in Blood Group*								*Total Number of Persons*	*Percentage of Non-secretors*
	O		*A*		*B*		*AB*			
	Secretors	*Non-secretors*	*Secretors*	*Non-secretors*	*Secretors*	*Non-secretors*	*Secretors*	*Non-secretors*		
M	26	13	11	18	0	3	1	0	72	47·2
F	3	3	1	2	0	2	0	0	11	63·6

Mean relative incidence among non-secretors compared with secretors:—
1 Comparison between stomal ulcer patients and controls:
χ^2 for difference from unity = 27·922, $n = 1$, $P < 0.001$
Fiducial limits of mean relative incidence, 2·47 : 1 and 7·20 : 1
χ^2 for homogeneity = 4·550, $n = 3$, $0.2 < P < 0.3$
2 Comparison between stomal ulcer and duodenal ulcer patients:
χ^2 for difference from unity = 4·446, $n = 1$, $P = 0.04$
Fiducial limits of mean relative incidence, 1·04 : 1 and 2·85 : 1
χ^2 for homogeneity = 5·365, $n = 3$, $0.1 < P < 0.2$

TABLE VII

NUMBER OF SECRETORS AND NON-SECRETORS AMONG PATIENTS WITH GASTRIC CANCER OR DIABETES BY SEX AND BLOOD GROUP IN COLLECTED SERIES

Series	*Sex*	*Number of Persons in Blood Group*								*Total Number of Persons*	*Percentage of Non-secretors*
		O		*A*		*B*		*AB*			
		Secretors	*Non-secretors*	*Secretors*	*Non-secretors*	*Secretors*	*Non-secretors*	*Secretors*	*Non-secretors*		
Carcinoma of stomach											
McConnell (1961a, 1961b)	M	51	21	44	16	4	3	1	0	140	28·6
	F	30	17	17	9	11	4	1	0	89	33·7
Newman *et al.* (1961)	M	26	9	29	7	5	2	2	1	81	23·5
	F	8	0	18	7	3	0	1	0	37	18·9
Present series	M	22	7	25	6	10	4	2	0	76	22·1
	F	9	6	8	3	2	1	0	0	29	34·5
Diabetes											
McConnell (1961a, 1961b)[1]	M	43	12	84	27	14	4	2	4	190	24·7
	F	39	17	44	15	6	2	3	2	128	28·1
Present series	M	11	3	19	2	3	0	2	0	40	12·5
	F	20	5	18	6	9	0	3	1	62	19·4

[1]Data not random for blood group.

TABLE VIII

INCIDENCE OF CARCINOMA OF STOMACH AND DIABETES MELLITUS AMONG NON-SECRETORS RELATIVE TO THAT AMONG SECRETORS' DATA FROM TABLES III AND VII

Disease Group	*Relative Incidence among Non-secretors*		*Difference of Mean Value from Unity*			*Homogeneity of Data*		
	Mean Value	*95% Confidence Limits*	χ^2	*n*	*P*	χ^2	*n*	*P*
Carcinoma of stomach	1·27:1	1·01:1 and 1·60:1	3·956	1	0·05	7·984	15[1]	0·90<P<0·95
Diabetes mellitus	1·05:1	0·81:1 and 1·37:1	0·147	1	0·7<P<0·8	7·616	9[1]	0·5<P<0·7

[1]Because of the small numbers in some of the groups it was necessary to combine into one group all the data for carcinoma of the stomach in women reported by Newman *et al.* (1961) and to combine the data in the present series for patients with diabetes in blood groups A, B, and AB.

control series given in Table III, it appears that the incidence of both conditions is also somewhat higher among non-secretors than among secretors. For carcinoma of the stomach the excess is about 27% and this difference is on the border of statistical significance (P = 0·05). For diabetes the excess is only 5% and the true incidence may well be independent of secretor status. The results of the statistical analysis of these data are summarized in Table VIII.

DISCUSSION

The large amount of information now available shows that stomal, duodenal, and gastric ulcers are all commoner in persons belonging to blood group O than in persons belonging to the other three blood groups. The excess incidence is greatest for stomal ulcer (80%), next greatest for duodenal ulcer (38%), and least for gastric ulcer (19%) (Roberts, 1957; Doll, Swynnerton, and Newell, 1960). More recently information has been obtained about the proportions of patients who secrete the blood group substances into their gastrointestinal tracts. The results, analysed in the present paper, show that the incidence of all three types of peptic ulcer is greater among non-secretors than among secretors and that the order of the relationship with non-secretion is the same as that with blood group O. The excess incidence is greatest for stomal ulcer (322%), next greatest for duodenal ulcer (80%), and least for gastric ulcer (42%).

The estimates of the incidence of duodenal ulcer and of gastric ulcer among non-secretors are based on large numbers of patients examined at three separate centres and can be regarded as reliable within fairly narrow limits. The figure for stomal ulcer is, however, open to doubt; the numbers are small and the results of the only other published series suggest that the true incidence may be considerably lower. Wallace *et al.* (1958) reported one non-secretor among four patients with a stomal ulcer belonging to groups A, B, or AB (25%), and 20 non-secretors among 48 patients with a stomal ulcer belonging to group O (42%). The corresponding proportions of non-secretors among duodenal ulcer patients were 37% and 40% and among control subjects 24% and 29%. In A, B, and AB subjects the presence of the blood group substances in the saliva was tested for directly by using group specific antibodies and the results should be comparable

with those in the present series. In the O subjects, however, non-secretion was determined indirectly by seeing whether the blood cells reacted with anti-Le^a serum. Hence the proportion of non-secretors may have been underestimated, as a result of excluding non-secretors who were Lewis negative.

The explanation of the relationship between group O, non-secretion, and peptic ulcer is not evident. The simplest explanation would be that the presence of the mucopolysaccharide blood group substances in the gastrointestinal secretions helped to protect the mucosa from digestion by the gastric juice and that the A and B substances were more efficient in this respect that the substances secreted by persons belonging to blood group O (Aird, Bentall, Mehigan, and Roberts, 1954). If this were so the blood group effect should be found only among secretors, whereas an equally strong effect has been found among non-secretors (Clarke *et al.*, 1959). This observation is confirmed by the present data. From the figures given in Tables III and IV it can be shown that the mean relative incidence of duodenal ulcer among persons with blood group O compared with that among persons with blood groups A, B, or AB is 1·48 : 1 for secretors and 1·47 : 1 for non-secretors; in both cases the relative incidence is significantly different from unity ($P < 0{\cdot}001$ and $P = 0{\cdot}001$ respectively). The two estimates are practically identical and it can be concluded with confidence that the effect associated with the different blood groups does not depend upon the presence of the blood group substances in the gastrointestinal secretions. Moreover, the fact that the relative incidences among group O subjects are so similar in secretors and in non-secretors suggests that the risks associated with the two factors are multiplicative. That the risks are multiplicative and not additive can be shown from the data in Tables III and IV in the following way. In the first case, the number of duodenal ulcer subjects expected among group O non-secretors would be approximately equal to $D_{AS} \times \frac{C_{ON}}{C_{AS}} \times p_o p_n$, where

D_{AS} = the number of group A, B, or AB secretors among the DU patients,

C_{AS} = the number of group A, B, or AB secretors among the control subjects,

C_{ON} = the number of group O non-secretors among the control subjects,

p_o = the relative incidence of DU among group O secretors compared with that among group A, B, or AB secretors, and

p_n = the relative incidence of DU among group A, B, or AB non-secretors compared with that among group A, B or AB secretors.

If, however, the risks were additive, the number of duodenal ulcer subjects expected would be approximately equal to:

$$D_{AS} \times \frac{C_{ON}}{C_{AS}} \times (p_o + p_n - 1).$$

The numbers expected on each hypothesis were calculated separately for men and for women in each of the three series and the results summed. On the first hypothesis the total number of group O non-secretors expected among the DU subjects was 336·1; on the second hypothesis the expected number was 286·5. The number actually observed was 340. Clearly the data are compatible with the risks being multiplicative ($0{\cdot}8 < P < 0{\cdot}9$); but it is unlikely that they would have been obtained if the risks had been additive ($P < 0{\cdot}01$). It seems reasonable, therefore, to conclude that the mechanisms by which blood group O and non-secretion affect the risk of developing a duodenal ulcer are related to one another, although they do not depend on the presence of the blood group substance in the secretions.

Analysis of the data for gastric ulcer, shown in Table V, gives a similar result. If the special risk among persons belonging to blood group O held only for secretors, the expected number of group O non-secretors among gastric ulcer patients would be 64·2; if the risks were additive, the expected number would be 79·9; if the risks were multiplicative, the expected number would be 88·7. In fact, the observed number of group O non-secretors was 96. The results clearly accord best with the last hypothesis.

In contrast to these findings the data for stomal ulcer, shown in Table VI, suggest that for this condition the type of ABO blood group may be unimportant among non-secretors. The numbers are, however, small and the evidence does not weigh heavily against the large body of evidence obtained for patients with gastric or duodenal ulcers. A different mechanism may be involved in the development of stomal ulcer; but it is not possible to reach a definite conclusion on this point until more data are available.

The data for gastric cancer and for diabetes mellitus suggest that there may be a small increase in the risk of the former condition among non-secretors but that the latter condition is independent of ABH secretion. Although there is some doubt about diabetes, it is probable that both conditions are commoner among persons belonging to group A than among persons belonging to group O and it might be anticipated that they would also differ from peptic ulcer in being commoner among secretors. This, however, is clearly not so and it

seems probable that the mechanism by which blood group A predisposes to gastric carcinoma and diabetes mellitus differs from the mechanism by which blood (group O) predisposes to peptic ulcer.

We are most grateful to Dr. C. A. Clarke and Dr. R. B. McConnell for providing us with unpublished data and for allowing us to refer to them, to Dr. J. Lennard-Jones for permitting us to include data obtained by him on the secretor status and ABO blood group of 70 of the duodenal ulcer patients studied at the Central Middlesex Hospital, and to Dr. P. Armitage for suggesting the method of statistical analysis described in the Appendix.

REFERENCES

Aird, I., Bentall, H. H., Mehigan, J. A., and Roberts, J. A. F. (1954). The blood groups in relation to peptic ulceration and carcinoma of colon, rectum, breast, and bronchus. *Brit. med. J.*, **2**, 315-321.

Boyd, W. C., and Shapleigh, E. (1954). Separation of individuals of any blood group into secretors and non-secretors by use of a plant agglutinin (lectin). *Blood*, **9**, 1195-1198.

Clarke, C. A., Edwards, J. W., Haddock, D. R. W., Howel-Evans, A. W., McConnell, R. B., and Sheppard, P. M. (1956). ABO blood groups and secretor character in duodenal ulcer. *Brit. med. J.*, **2**, 725-731.

——, Evans, D. A. P., McConnell, R. B., and Sheppard, P. M. (1959). Secretion of blood group antigens and peptic ulcer. *Ibid*, **1**, 603-607.

Discombe, G., and Meyer, H. (1952). The capillary-tube method of rhesus testing. *Amer. J. clin. Path.*, **22**, 543-548.

Doll, R., and Drane, H. (1961). To be published.

——, Swynnerton, B. F., and Newell, A. C. (1960). Observations on blood group distribution in peptic ulcer and gastric cancer. *Gut*, **1**, 31-35.

Glynn, A. A., Glynn, L. E., and Holborow, E. J. (1959). Secretion of blood-group substances in rheumatic fever. *Brit. med. J.*, **2**, 266-270.

McConnell, R. B. (1961a). The mechanism by which blood groups antigens influence gastrointestinal disorders. *Proc. int. Congr. Gastroenterology, Leyden*, 1960, pp. 41-45.

—— (1961b). Personal communication.

Newman, E., Naifeh, G. S., Auer, J. E., and Buckwalter, J. A. (1961). Secretion of ABH antigens in peptic ulceration and gastric carcinoma. *Brit. med. J.*, **1**, 92-94.

Roberts, J. A. F. (1957). Blood groups and susceptibility to disease. A review. *Brit. J. prev. soc. Med.*, **11**, 107-125.

Wallace, J., Brown, D. A. P., Cook, I. A., and Melrose, A. G. (1958). The secretor status in duodenal ulcer. *Scot. med. J.*, **3**, 105-109.

Woolf, B. (1955). On estimating the relation between blood group and disease. *Ann. hum. Genet.*, **19**, 251-253.

APPENDIX

Let the numbers of non-secretors and of secretors in the disease series be h and k and in the corresponding control series be H and K. Then the incidence of the disease among non-secretors relative to that among secretors is hK/Hk.

With Woolf's (1955) notation, let

$x = hK/Hk$,

and $y = \log_e x$;

then V(y), the variance of y, $= \left(\frac{1}{h} + \frac{1}{k} + \frac{1}{H} + \frac{1}{K}\right)$.

Let $w = 1/V(y)$,

and Y = the logarithm of the mean relative incidence of the disease among non-secretors for all the various groups examined.

Then $Y = Swy/Sw$

and χ^2, for the difference of the mean relative incidence from unity, $= (S\ wy)^2/Sw$ with one degree of freedom and χ^2, for the homogeneity of the groups, $= Swy^2 - (Swy)^2/Sw$, with degrees of freedom one less than the number of groups.

The homogeneity of the results for men and for women may be tested separately as follows:

Let y_m be the value of y obtained for men in any one blood group at one centre, and y_f be the corresponding value for women.

Let $z = y_f - y_m$

Then V(z), the variance of z, $= V(y_f) + V(y_m)$

Let $w_z = 1/V(z)$

Then $\bar{z}$, the weighted mean of z, $= S\ w_z\ z/Sw_z$

and χ^2, for the difference of $\bar{z}$ from zero, $= (Sw_z z)^2/Sw_z$ with one degree of freedom.

The homogeneity of the results for the three centres may be tested as follows:

Let y_1, y_2, and y_3 be the values of y obtained at each of the three centres for one sex and one blood group.

Then $\bar{y}_1 = Sy_1/6$,

$V(y_1) = S[V(y_1)]/36$,

and $W_1 = 1/V(\bar{y}_1)$.

Then χ^2, for homogeneity, $= SW_1\bar{y}_1^2 - \frac{S(W_1\bar{y}_1)^2}{SW_1}$ with 2 degrees of freedom.

The homogeneity of the results for the three principal blood groups may be tested in the same way as the results for the three centres.

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Experiences with extraperitoneal colostomy and ileostomy

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SYNOPSIS A technique for extraperitoneal colostomy and ileostomy has been used by one of the authors since 1952. The technique is described and the results reviewed.

The small intestine may become obstructed after colostomy or ileostomy, due to its proiapsing through the lateral space, and, to avert this possibility, it is a common practice to close the space by suturing the terminal bowel to the parietal peritoneum. Sames (1958) and Goligher (1958) have suggested that the formation of this space may be avoided by bringing the terminal colon or ileum to the surface through the extraperitoneal tissues. A similar procedure has been used by one of us since 1952, and we feel that our experience over a number of years shows that this method of forming colostomies and ileostomies does not result in any additional immediate or late complications. We therefore consider it worth recording our results in support of the previous writers.

FORMATION OF THE EXTRAPERITONEAL COLOSTOMY

An incision is made in the parietal peritoneum immediately lateral to the lower end of the descending colon. Usually this incision can be a small extension upwards of the incision already made for the mobilization of the sigmoid colon. With a long artery forceps on the edge of the peritoneum, the anterior flap is raised and with two fingers of the right hand a tunnel is easily formed in the retroperitoneal tissues and extended round to the deep surface of the anterior abdominal wall (Fig. 1). A disc of skin 1 in. in diameter is removed from the abdominal wall where the colostomy opening is to be placed, usually at the lateral border of the rectus muscle on a line between the umbilicus and the left anterior superior spine. The muscles deep to the skin opening are incised so as to enter the extraperitoneal tunnel which had just been formed. Long sponge forceps are then passed through the skin opening along the tunnel and so into the peritoneal cavity. The de Martel clamp on the upper end of the previously divided sigmoid colon is grasped by the sponge forceps and is brought out through the extraperitoneal tunnel so that it and a short length of colon lie on the abdominal wall (Fig. 2).

A few catgut stitches are inserted to fix the upper leaf of peritoneum to the colon as it passes extraperitoneally, and in particular to obliterate any small gap that may be present on either side of the colon.

Finally, after the abdomen has been closed, any excess of colon on the skin surface is cut away and the bowel mucosa sutured to the skin edge.

FORMATION OF THE EXTRAPERITONEAL ILEOSTOMY

The extraperitoneal ileostomy is fashioned in a similar manner by starting the retroperitoneal tunnel in the right iliac fossa just below the lowest point of the small bowel mesentery. The clamped and divided terminal ileum is then drawn through the tunnel on to the skin surface. The same technique is used in bringing an ileal bladder to the skin surface.

RESULTS

Since 1952, 51 extraperitoneal colostomies and six extraperitoneal ileostomies have been fashioned. Twenty-two of the former have been made in the last 18 months and have functioned without complications. We have reviewed those fashioned before 1959 to see whether any late complications occurred that were attributable to this operation.

EXTRAPERITONEAL COLOSTOMY This operation was performed on 29 patients before 1959; 27 suffered from carcinoma of the rectum, one patient with carcinoma of the bladder was treated by total cystectomy and colostomy with transplantation of the ureters into a rectal bladder, and a further patient with incontinence of faeces due to prolapse of the rectum, which previous surgery had failed to cure, was made comfortable with a left iliac colostomy.

There were three post-operative deaths, and post-mortem examination showed these to be due to cerebral thrombosis, salmonella enteritis, and ileus

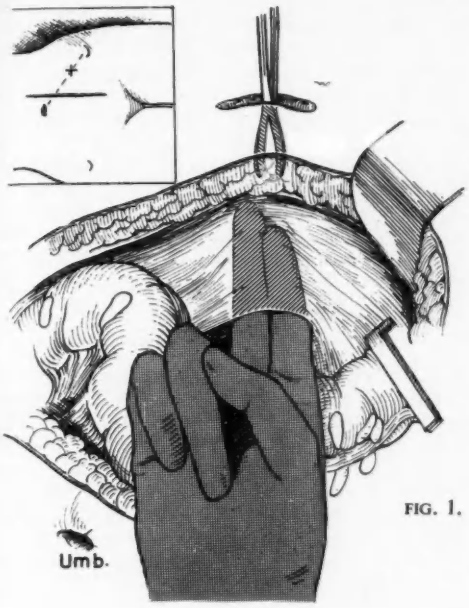

FIG. 1.

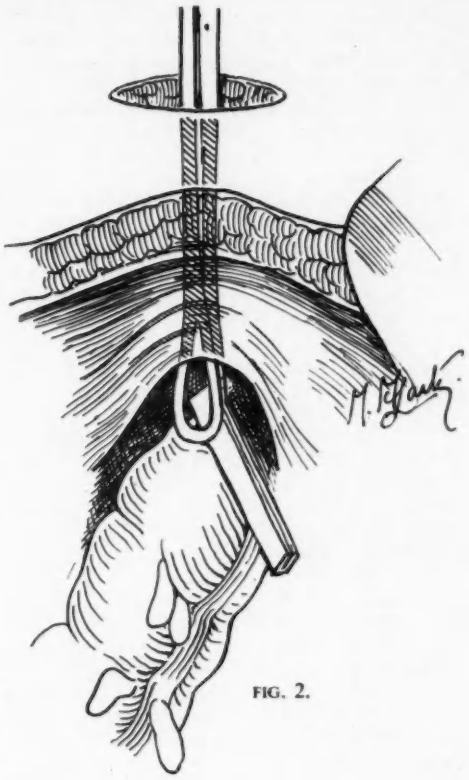

FIG. 2.

FIG. 1. *The formation of the retroperitoneal tunnel.*

FIG. 2. *Method of passing the crushed and divided bowel through the tunnel.*

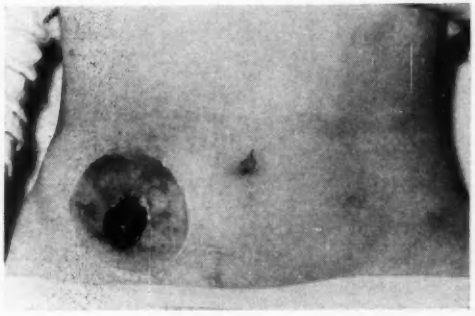

FIG. 3. *Ileostomy eight years after operation.*

following laparotomy for post-operative obstruction, which was due to adhesions between the small bowel and the pelvic suture line.

Of the remaining 26 patients, 10 are alive and well, 12 have died from their original disease, and four from other causes. Half died in hospital and half at home under the care of their own doctors, who kindly gave us details of their last days.

The average time the colostomies of the deceased patients functioned was over two years. The remainder are still satisfactory after an average of three years and seven months; one of these was fashioned over seven years ago.

Two colostomies became stenosed at the mucocutaneous junction due to scarring. Another was excised *en bloc* for local recurrence of growth after two years, and replaced by a similar colostomy which functioned for over a year without trouble. In only one case was there prolapse of the mucosa; this was slight and did not require surgery.

EXTRAPERITONEAL ILEOSTOMY Six patients with ulcerative colitis have been treated by total colectomy. Two of these seriously ill patients died post-operatively and necropsy showed that the ileostomies were uncomplicated. The remaining four patients have been alive for over seven years. One has since had a laparotomy for obstruction due to adhesions, at which 10 in. of gangrenous small bowel was resected. The ileostomy continues to function well, although it has had to be digitally dilated (Fig. 3). There has been no case of prolapse of small bowel through the ileostomy.

DISCUSSION

Closure of the lateral space may be difficult and if non-absorbable sutures are used there is a risk of a troublesome sinus forming. We have recently seen a case in which an ileostomy was formed and the lateral space closed with catgut, but on a second laparotomy small bowel was found to have prolapsed through the space, which admitted three fingers. This has convinced us that closure of the lateral space with sutures may not be permanent however carefully it is done. The extraperitoneal route does not result in the formation of a lateral space and thus one cause of post-operative obstruction is avoided.

Our experience drawn from this series leads us to believe that the extraperitoneal route of the bowel does not result in any additional complications. Goligher's suggestion that this method may reduce the incidence of prolapse, as the bowel may be better fixed by its oblique exit from the abdomen, is supported by the fact that there has been no case of prolapse requiring surgery in this series. The extraperitoneal tissues do not contract and cause stenosis of the bowel; the only stenosis has been at the skin surface. One ileostomy, complicated by post-operative abscess formation in the surrounding tissues, has functioned without stenosis for over six years. Secondary spread of growth was seen surrounding the last 6 in. of colon in another patient, but the colostomy remained open until the patient's death.

CONCLUSION

We conclude that the extraperitoneal method of forming a colostomy or an ileostomy is an effective method of avoiding the dangers associated with the formation of a lateral space. The oblique exit of the bowel through the abdominal wall appears to reduce the likelihood of prolapse without resulting in any long-term stenosis of the intramural bowel.

We agree with Sames and Goligher that this operation is satisfactory and have adopted it as our standard technique.

REFERENCES

Goligher, J. C. (1958). Extraperitoneal colostomy or ileostomy. *Brit. J. Surg.*, **46**, 97-103.
Sames, C. P. (1958). Extraperitoneal colostomy. *Lancet*, **1**, 567-568.

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The incidence and pattern of peptic ulcer in Indians and Africans in Durban

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SYNOPSIS This paper records the hospital incidence of peptic ulcer amongst Africans and Indians in Durban and draws attention to the lower incidence amongst Africans.

Peptic ulcer symptoms are amongst the commonest complaints in Indian patients in Durban; epigastric pain related to meals, dyspepsia, and periodic exacerbations of such pain frequently form a secondary complaint when the chief complaint is referable to other parts of the body. Diseases of the stomach and duodenum, on the other hand, are relatively much less frequent in African patients.

The frequency and types of peptic ulcer among Indians and Africans admitted to this hospital in the 10-year period 1950-59 inclusive have been analysed. No separate study has been made of factors such as diet, social adjustments, or emotional stress which might influence the incidence of the disease. For comparison the incidence of peptic ulcer in White patients at Addington Hospital during a two-year period has been noted. Only those cases have been included in which a proven diagnosis of peptic ulcer has been made either at operation, necropsy, or on positive radiological evidence in association with a characteristic history of peptic ulcer or one of its complications.

Seven hundred and fifty-seven records of proven peptic ulcer were available at King Edward VIII Hospital. Six hundred and fifty-seven peptic ulcers occurred in Indians and 100 in Africans. In this period there were 65,000 Indian and 361,000 African admissions excluding maternity cases. Thus of every 1,000 Indian admissions 10·0 were for peptic ulcer while the rate per 1,000 African admissions was 0·28. In a two-year period at Addington Hospital of 16,230 in-patients, 183 (Table I) with peptic ulcer were admitted (11·3 per 1,000).

TABLE I

INCIDENCE OF PEPTIC ULCER IN THREE RACIAL GROUPS

Race	*Period*	*No. of Cases*	*Total Admissions*	*Incidence per 1,000 Admissions*	*Ratios*	*Indian*	*African*	*White*
						(657 cases)	*(100 cases)*	*(183 cases)*
Indian	(1950-59)	657	65,000	10·0	Male : female	8 : 1	8 : 1	3 : 1
African	(1950-59)	100	361,000	0.28	Duodenal : gastric	4·6 : 1	3·5 : 1	3·4 : 1
White	(1957-58)	183	16·230	11·3	Duodenal ♂ : ♀	7 : 1	6·8 : 1	3·6 : 1
					Gastric ♂ : ♀	15·9 : 1	21 : 1	1·8 : 1

TABLE II

NEOPLASMS OF STOMACH IN INDIANS AND AFRICANS (1950-59)

Indians			*Africans*		
Male : Female	*Total*	*Incidence per 1,000 Admissions*	*Male : Female*	*Total*	*Incidence per 1,000 Admissions*
61 25 = 2·4 : 1	86	1·03	49 26 (Carcinoma)	75	0·20
			5 1 (Sarcoma)	6	
			54 27 = 2 : 1	81	0·22

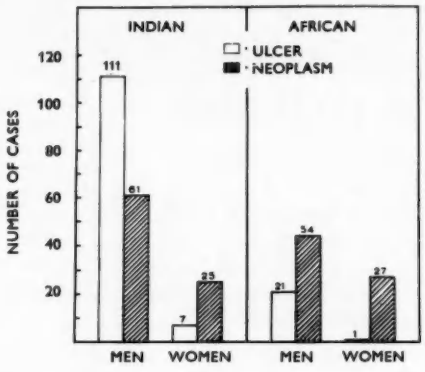

FIG. 1. *Incidence of ulcer and neoplasm in the stomach in Indians and Africans.*

In the same 10-year period there were admitted 86 Indians with carcinoma of the stomach, an incidence of 1·03 per 1,000 admissions, and 81 Africans with malignancy of the stomach (75 with carcinoma and six with lymphosarcoma), an incidence of 0·22 per 1,000 admissions and 0·20 when lymphosarcoma is excluded (Table II and Fig. 1).

Table I shows an analysis of the 657 Indian patients compared with the 100 Africans and 183 Whites. The ratio of duodenal ulcer to gastric ulcer was 4·6 : 1 in Indians, 3·5 : 1 in Africans, and 3·4 : 1 in Whites. The male-to-female ratio of duodenal ulcer was 7 : 1 in Indians, 6·8 : 1 in Africans, and 3·6 : 1 in Whites (Figs. 2 and 3). The male-to-female ratio of gastric ulcer was 11·9 : 1 in Indians while in the Africans only one gastric ulcer was found in a woman. In the White hospital group this ratio was 1·8 : 1.

Table III and Fig. 4 analyse the incidence of perforation and haemorrhage in the three racial groups. Duodenal perforation occurred approximately as frequently in Indians as in Africans and Whites (15·6%, 14%, and 15·5%). However, this was true for the males only. Duodenal perforation amongst Indian women was extremely uncommon, (3%) as it was in the White group, whereas in the small group of 10 African women in two the ulcer had perforated (20%).

The incidence of gastric perforation in men was high among Indians (32·4%), was common amongst

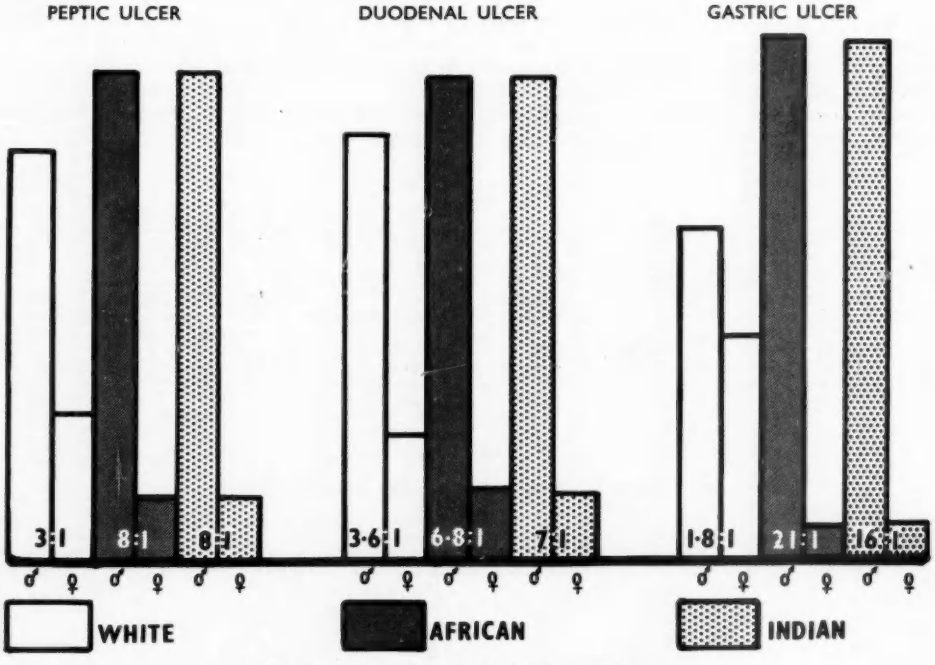

FIG. 2. *Sex ratios of peptic ulcer in three racial groups.*

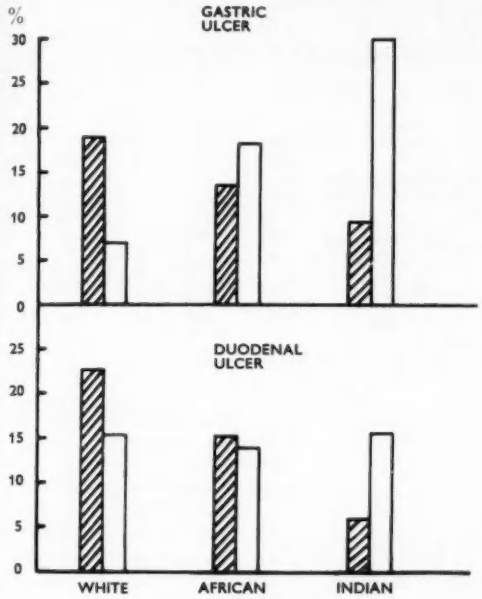

FIG. 3. *Incidence of complications of peptic ulcer in three racial groups. Hatched column denotes haemorrhage, and unhatched column perforation.*

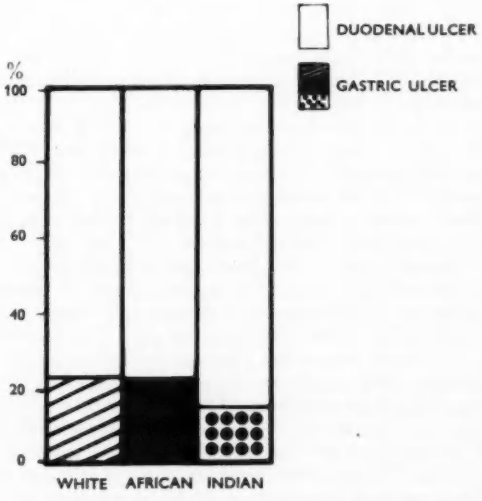

FIG. 4. *Ratio of duodenal to gastric ulcer in three racial groups.*

TABLE III

INCIDENCE OF COMPLICATIONS OF PEPTIC ULCER AS PERCENTAGE OF TOTAL NUMBER IN EACH GROUP

	Indians (118 cases)	*Africans (15 cases)*	*Whites (25 cases)*
Peptic perforations	18·0	15	13·6
Total duodenal perforations	15·6	14	15·5
♂ duodenal perforations	17·4	13·2	19
♀ duodenal perforations	3·0	20	3
Total gastric perforations	30	18·2	7
♂ gastric perforations	32·4	19	11
♀ gastric perforations	None	None	None
Peptic haemorrhage	6·5	14	22
Total duodenal haemorrhage	5·9	15·2	22·7
♂ duodenal haemorrhage	6·4	14·7	19
♀ duodenal haemorrhage	4·5	20	35
Total gastric ulcer haemorrhage	9·3	13·6	19
♂ gastric ulcer haemorrhage	9·0	14·3	18·5
♀ gastric ulcer haemorrhage	14·3	None	20

Africans (19%), and less so in Whites (11%). There were no gastric ulcer perforations in any of the women in the three groups.

The incidence of haemorrhage varied more than perforation in the three races. Both duodenal and gastric ulcers in the African male group were complicated by bleeding with approximately the same frequency (15·2% and 13·6%). Duodenal haemorrhage occurred in two women (20%). Amongst Indian patients haemorrhage from duodenal ulcer was relatively infrequent (5·9%) and approximately the same in both sexes but more common from gastric ulcer (9% and 14·3% in men and women). In White patients haemorrhage was a more frequent complication in both ulcer groups (18 to 22%), more particularly from duodenal ulcer in women (35%).

MORTALITY

Twenty-two deaths occurred amongst Indian patients with peptic ulcer (Table IV), five being caused by perforations found at necropsy and three patients dying from haemorrhage. These eight patients were not operated upon. The operative mortality in the Indian group was 7·1% for curative procedures, 3·5% for closure of perforated ulcers, these four being gastric ulcers. The overall mortality was 3·7%.

Eight African patients died without operation being done, four being shown at necropsy to have had perforations, three having bled, and one death being associated with a pancreatic tumour. Two (6·9%) died following curative surgery.

The details of operative treatment of peptic ulcer in the two five-year periods are set out in Table V.

TABLE IV

MORTALITY FROM PEPTIC ULCER (1950-59)

Indians

Cause of Death	*Mortality Rate*			
	cases			%
Curative surgery (including haemorrhage)	132	10	8 gastrectomy 1 gastro-jejunostomy 1 haemorrhage (duodenal ulcer)	=7·1[1]
Closure of perforations	115	4	(all gastric ulcer)	=3·5
Perforation at necropsy	5	5	(4 duodenal and 1 gastric ulcer	=7·5
	120	9		
Haemorrhage (not operated on)		3		
		22		

Overall mortality = 3·7% (22 of 657 cases)

Africans

Cause of Death	*Mortality Rate*			
	cases			%
Curative surgery (including haemorrhage)	29	2		=6·9[1]
Closure of perforation	11	—		
Perforation at necropsy	4	4	(3 gastric and 1 duodenal ulcer)	=27
	15	4		
Haemorrhage (not operated on)		3		
Gastric ulcer with pancreatic tumour		1		
		10		

Overall mortality = 10% (10 of 100 cases)

[1]Second five-year mortality following curative surgery = 4·3%

TABLE V

OPERATIVE TREATMENT OF PEPTIC ULCER (1950-59)

Indians

	Total Operations	*Closure of Perforation*	*Curative Operation*
Duodenal ulcer ♂	173 (24)[1]	78 (16)	95 (8)
Duodenal ulcer ♀	12 (1)	2 (–)	10 (1)
Gastric ulcer ♂	60 (16)	35 (9)	25 (7)
Gastric ulcer ♀	2 (1)	— (–)	2 (1)
Totals	247 (42)	115 (25)	132 (17)

Africans

	Total Operations	*Closure of Perforation*	*Curative Operation*
Duodenal ulcer ♂	29 (5)	9 (1)	20 (4)
Duodenal ulcer ♀	5 (1)	2 (–)	3 (1)
Gastric ulcer ♂	10 (2)	4 (1)	6 (1)
Gastric ulcer ♀	— (–)	— (–)	— (1)
Totals	49 (8)	15 (2)	29 (7)

[1]The figures in brackets represent the first five-year period.

AGE

The highest incidence of duodenal ulcer among Indian males was in the 20- to 29-year group (47·5%). Sixty-nine per cent occurred in the group aged 20 to 39 years and 83% in the group aged 20 to 49 years. Indian women had duodenal ulcer equally in the three decades 20 to 49 years, this period accounting for 75% of duodenal ulcers (Table VI).

Gastric ulcer among Indian men also occurred equally in the three decades 20 to 49 years (77% occurring in this period). In six Indian women gastric ulcers occurred four times between the ages of 30 and 49 years and twice in the 10-to-19 age group.

Among Africans the highest incidence of duodenal ulcers in men was in the 30- to 39-year group (35%) with 22% occurring in each of the 20- to 29- and 40- to 49-year groups, 79% occurring in the 20- to 49- year age groups. Gastric ulcer in males also occurred most commonly in the 30- to 39-year group (47%), 73% occurring between the ages of 30 and 49 years.

Of the 82 duodenal perforations in Indian men, 50% occurred in the age group 20 to 29, and of 36 gastric perforations in Indian men, 90% were equally spread over each of the three decades (20 to 49 years). The number of perforations among Indian women was too small to be analysed.

In African men six of the nine duodenal perforations occurred between the ages of 20 and 39 years, while of the four gastric perforations in men, two occurred in the 30- to 39-year age group.

The distribution of haemorrhages in age groups in each racial group was approximately the same as the frequency distribution of ulcers.

DISCUSSION

Both groups of patients of King Edward VIII Hospital are drawn from a generally poor and malnourished community. The dietary habits of Indians

TABLE VI

INCIDENCE OF PEPTIC ULCER IN DIFFERENT AGE GROUPS (INDIANS AND AFRICANS)

		Age (years) 0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79
		Age (years)							
Indian									
Duodenal ulcer	Male	1 (0·2)[1]	26 (5·8)	209 (47·5)	95 (21·6)	61 (14·7)	24 (5·4)	23 (5·2)	1 (0·2)
	Female	—	7 (11·7)	15 (25)	16 (27·3)	14 (23·3)	7 (11·6)	1 (1·7)	—
Gastric ulcer	Male	—	5 (5)	25 (25)	28 (28)	24 (23·3)	9 (9)	6 (6)	3 (3)
	Female	—	2 (33)	—	2 (33)	2 (33)	—	—	—
African									
Duodenal ulcer	Male	—	6 (9·2)	15 (22)	24 (35·3)	15 (22)	7 (10·2)	1 (1·5)	—
	Female	—	—	2 (22)	2 (22)	1 (11)	3 (33)	1 (11)	—
Gastric ulcer	Male	—	—	2 (10·5)	9 (47)	5 (26)	3 (15·8)	—	—
	Female	—	—	—	1 (100)	—	—	—	—

[1]Percentage incidence in brackets.

and Africans have in common only a poor protein intake; generally the African subsists largely on a high carbohydrate diet, whereas the Indian eats in addition a great deal of vegetable fat.

The frequency of peptic ulcer amonst Indians is partly reflected in the figures for admissions to this hospital in a 10-year period. What is not reflected is the frequency of out-patient attendances for symptoms of peptic ulcer which are treated by diet and drugs. These represent one of the commoner complaints amongst the Indian population.

The infrequency of peptic ulcer in Africans is seen in the figures in Table I. Despite an improved hospital service by increase in staff and radiological facilities, the rate of admission of Africans with this disease has not increased out of proportion to the in-patient increase in the past five years. In the first five-year period the average annual Indian admission rate for peptic ulcer was 48 and the African seven. In the second five-year period the figures were 84 and 13 respectively; during this period there has been an increase of 80% in Indian and of 40% in African admissions. The bulk of the African patients admitted to this hospital are drawn from the urban and periurban area of Durban with a minority from outlying rural districts. In a recent hospital survey (University of Natal, 1958), 80% of all out-patients were from the Durban area; among low-paid workers, between 20 and 39 years old in five Durban industries, 55% had lived in Durban 10 years or more and only 11% had been in an urban environment for less than five years. Better paid workers and more settled family groups probably include still fewer migrant labourers and persons resident here for less than five years. While no accurate estimate has been made of the effect of work or change in diet in the series of Africans with peptic ulcer, the incidence of the condition in this area does not appear to confirm the view that the rapid urbanization of the African is reflected by an equally rapid increase of this disease (Baldachin and Palmer, 1959; Carter, 1960; Esser and Coetzee, 1960; Gelfand, 1957). What has increased is an awareness of the clinical condition in Africans; the disease has been seen sufficiently often to have impressed clinicians that it is not a rarity and must be considered in cases with upper abdominal symptoms. A considerable number of the diagnoses in the first five-year period were made at necropsy whereas latterly the overwhelming majority have been clinically confirmed.

The admission rate for patients with peptic ulcer is, however, diluted here by the large proportion of patients admitted for urgent surgery necessitated by trauma (approximately 40% of African surgical cases) as well as by a large gynaecological and paediatric unit. Thus, if these categories of patients are excluded, the incidence of admissions for peptic ulcer to general medical and surgical beds is considerably higher than those indicated, particularly amongst the Africans and to a lesser extent amongst the Indians.

The admission rate of patients with cancer of the stomach reveals an interesting difference. The incidence per 1,000 Indian admissions was 1·03 compared with a figure of 10 for peptic ulcer. The African incidence was 0·22 (0·20 excluding sarcomata), almost the same as for ulcer (0·28). Thus cancer of the stomach occurred almost as frequently as peptic ulcer in Africans in this area, whereas in Indians it occurred eight times less frequently. The incidence of gastric ulcer and gastric neoplasm in the two racial groups is seen in Fig. 1. There was a considerably higher rate of cancer in both African sexes and in Indian women but not in Indian men. Of the malignancies occurring in Africans, 7·5% were lymphosarcomata. Despite the difference in admission rates for the two races to this hospital and

those reported by Esser and Coetzee (1960), the figures show a similar trend.

Peptic ulcer in Indians is a disease predominantly seen in the early twenties, many patients having histories dating from their teens. In the African the peak incidence of duodenal and gastric ulcer is in the 30- to 39-year group. The incidence of complications of peptic ulcer in Indians is strikingly different from that of the White population and of the Africans. In the Indian group 17·4% of male patients with duodenal ulcers and 32·4% of male patients with gastric ulcers were admitted for perforation. Duodenal perforations in women were a rarity and no gastric ulcers in women have been seen with perforation. Haemorrhage, on the other hand, is not a common complication in the Indian group (5·6% in duodenal ulcers and 10% in gastric ulcers in men) while in a small group of women the corresponding figures were a little higher (4·5% and 14·3%). Pyloric stenosis in varying degree is a frequent complication.

Fourteen per cent of duodenal ulcers in African men are perforated and 15·2% caused haemorrhage. Of gastric ulcers in African men, 18·2% had perforated and 14·3% had bled. A small series of duodenal ulcers in African women showed that 20% had perforated and 20% had bled. There was only one gastric ulcer in African women, a large crater in the lesser curvature which at operation appeared to be inoperable carcinoma but at necropsy was shown to be a peptic ulcer. The rarity of gastric ulcer in African women is also borne out in the series of 58 ulcers reported by Esser and Coetzee (1960) who found only one in this group. With the exception of gastric ulcer in women these figures for the complications of ulcer in Africans accord more closely with those for White patients in this area (Fig. 4).

In discussing the incidence of peptic ulcer or any other disease among black people the use of the word 'African' may be as misleading as the use of the word 'European' on the Continent of Europe. The types of people in southern and central Africa differ in their physique, state of health, degree of education, and city living and dietary habits no less than those extending in Europe from Spain to Scandinavia. Moreover, the bulk of the medical and surgical conditions in an African general hospital in the Union as well as in other parts of Africa, comprise trauma, sepsis, malnutrition, tuberculosis, amoebiasis, and acute pulmonary infections; until these conditions cease to be the overwhelming public health and social problems that they are, and therefore their victims cease overcrowding the hospitals, the non-tropical diseases of well-nourished people must perforce be relegated to a position of secondary importance except in severe cases.

Raper (1958) has shown the high necropsy incidence of ulcer in Uganda, where Shaper and Williams (1959) agree that ulcer is a well-recognized clinical entity; although usually occurring in the labouring and farming people it is a not infrequent diagnosis in university students (19 of 400 students). Konstam (1954) and others (Joly, 1956) have repeatedly pointed out the frequency of peptic ulceration in West Africa. Humphries (1959), on the other hand, points out the rarity of ulcer in Nyasaland.

In Sierra Leone, Carter (1960) and Baldachin and Palmer (1959) comment on their impression of the increasing frequency of peptic ulcer among those African groups whose standard of living has increased.

In South Africa, Eagle and Gillman (1938) noted the rarity of ulcer at necropsy in Africans in Johannesburg; Charlewood and Frylinck (1951) reported an 0·09% incidence in non-White hospitals in Johannesburg, and McKenzie (1957) an incidence in a two-year period at King Edward VIII Hospital in Durban of 0·068%. The incidence of 0·025% of hospital admissions in the present series is still lower; in the first five-year period the admission rate was 0·021% and in the second five-year period only 0·03%.

The ratio of duodenal to gastric ulcer here is 3·3 : 1, similar to the ratio for White and Indian patients. The rarity of gastric ulcer among Africans reported by Ellis (1948) and Joly (1956) in Nigeria, and Shaper and Williams (1959) and Raper (1958) in Uganda, is borne out in women in Durban where only a single case has been seen; 21 of 22 gastric ulcers occurred in men.

The sex ratio of peptic ulcer varies in different parts of Africa. In Nigeria, Ellis (1948) reported a 9·3 : 1 ratio for duodenal ulcers whereas Aitken (1933) reported a 12 : 1 ratio and Joly (1956) one of 3·7 : 1. The present series shows a 8 : 1 ratio, being 6·8 : 1 for duodenal ulcer and 21 : 1 for gastric ulcer. These figures are all uncorrected for the admission rate of men and women, which, in this hospital, is approximately 1·6 : 1. The corrected rate here for duodenal ulcer is 3·6 : 1.

The incidence of the complications of peptic ulcer throughout Africa appears to vary widely (Kark, 1959). In Nigeria (Joly, 1956), haemorrhage and perforations occur in 2% and 0·1% of cases respectively. This is the same pattern as in India (Somervell and Orr, 1936) where the figures are 0·1% and 1%, and among the Swahili of Zanzibar (Vassallo, 1937). The White patients in the Union suffer the complications with about the same frequency as do those in Britain; the Indians have a low incidence of haemorrhage and a high incidence of perforation; the Africans in Durban have an

overall rate of perforation and bleeding not dissimilar to that of the Whites, notable differences being the considerably higher rate of gastric perforations in men and the rarity of gastric ulcer and therefore its complications in women.

OPERATIVE TREATMENT

Table IV summarizes the mortality following emergency and elective surgery for peptic ulcers. The increase in elective operations reflects the increase in staff and facilities. The mortality for curative surgical operations, including those for haematemesis, was 7·1% and 6·9% in Indians and Africans respectively. This high figure is partly accounted for by the considerable overcrowding and inadequate nursing and medical personnel during the first five-year period; in the second five-year period the mortality for elective surgery was 4·3% (six of 137 cases) and in 1959 was 1·6% (one of 62 cases). Table V compares the surgical treatment in the first five-year period with that in the second five-year period.

SUMMARY

The hospital incidence of peptic ulcer in Africans and Indians in Durban has been analysed. The material includes 757 proven cases admitted during 1950-59. The admission rate has been compared with the incidence of neoplasm of the stomach in each group (167 cases). For comparison a two-year incidence of peptic ulcer in Whites has been included (186 cases).

The peak age incidence for Indians with duodenal ulcer is 20 to 29 years and for Africans 30 to 39 years.

Attention is drawn to the low incidence of peptic ulcer in Africans, with the reservation that the figures must be viewed in relation to a total admission rate comprising cases mostly of trauma, of sepsis, and of acute infection.

Gastric ulcer in Indians is very uncommon and in women is rare; gastric ulcer in Africans is also very seldom seen, and, as in Indians, is rare in women, in White women it occurs half as frequently as in men.

There is a low incidence of duodenal perforation in women in all three racial groups, the African figure of 20% only representing two cases.

No gastric ulcer perforated in women in any of the three groups.

Haemorrhage complicating peptic ulcer is uncommon in the Indian group compared with the relatively high incidence in Africans and Whites. In this respect the low incidence in Indians is in close accord with the rarity of this complication in South India and in Nigeria. In Indians gastric ulcers appear more likely to bleed than do duodenal ulcers.

The mortality following elective and emergency surgery is briefly analysed.

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REFERENCES

Aitken, A. B. (1933). Remarks on peptic ulcer. *W. Afr. med. J.*, **6**, 63-65.

Baldachin, B. J., and Palmer, P. E. S. (1959). Peptic ulcer in Africans. *Brit. med. J.*, **2**, 304.

Carter, F. S. (1960). *Ibid*, **1**, 505-506.

Charlewood, G. P., and Frylinck, R. (1951). Some discrepancies in disease incidence between the European and the South African negro (Bantu). *S. Afr. med. J.*, **25**, 551-556.

Eagle, P. C., and Gillman, J. (1938). The incidence of peptic ulcer in the South African Bantu. *S. Afr. J. med. Sci.*, **3**, 1-6.

Ellis, M. (1948). A study of peptic ulcer in Nigeria. *Brit. J. Surg.*, **36**, 60-65.

Esser, H. R., and Coetzee, T. (1960). Peptic ulcer in Indians and Africans in Natal. *Med. Proc.*, **6**, 153-158.

Gelfand, M. (1957). *The Sick African*, 3rd ed. Juta, Cape Town.

Humphries, S. V. (1959). Peptic ulcer in Africans. *Brit. med. J.*, **2**, 1253.

Joly, B. M. (1956). Peptic ulcer in Western Nigeria and Southern United States. *W. Afr. med. J.*, n.s. **5**, 55-63.

Kark, A. E. (1959). Aetiology and demography of peptic ulcer, *Leech*, **29**, 117-130.

Konstam, P. G. (1954). Peptic ulcer in Southern Nigeria. *Lancet*, **2**, 1039-1040.

McKenzie, M. B. (1957). Peptic ulceration in the African of Durban. *S. Afr. med. J.*, **31**, 1041-1045.

Raper, A. B. (1958). The incidence of peptic ulceration in some African tribal groups. *Trans. roy. Soc. trop. Med. Hyg.*, **52**, 535-546.

Shaper, A. G., and Williams, A. W. (1959). Peptic ulcer in Africans. *Brit. med J.*, **2**, 757-758.

Somervell, T. H., and Orr, I. M. (1936). Some contributions to the causation, pathology, and treatment of duodenal ulcer and its complications. *Brit. J. Surg.*, **24**, 227-245.

Vassallo, S. M. (1937). Duodenal ulcer in Zanzibar. *E. Afr. med. J.*, **14**, 83-88.

University of Natal (1958). Department of Social and Preventive and Family Medicine: A Study of the King Edward VIII Hospital Out-Patient Service.

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The first recorded case of Whipple's disease?

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It is more than half a century since Whipple described a rare form of intestinal lipodystrophy, 'a hitherto undescribed disease characterized anatomically by deposits of fat and fatty acids in the intestinal and mesenteric lymphatic tissues' (Whipple, 1907). Some idea of the rarity of the condition may be gained from the fact that it was not until 1936 that the next acceptable case was published. The first generally recognized British case appeared only in 1938, under the title of 'mesenteric chyladenectasis', with no reference to Whipple's article (Glynn and Rosenheim, 1938).

It was of some historical interest, therefore, to discover a specimen of Whipple's disease in the pathological museum at Westminster Medical School, and to find that the case had been recorded in British medical literature some 12 years before Whipple published his paper.

It was during a recent reorganization of the gut section in the Westminster museum that an old specimen came under review. This was a U-shaped strip of tissue, gray with age, and with a finely papillated surface. It was labelled 'lymphangiectasis of the stomach', and the museum catalogue simply recorded that the patient was a man who had suffered from intermittent attacks of diarrhoea and vomiting in 1894, and died of exhaustion within a few months.

Closer inspection of the specimen revealed that this was not a stomach but a duodenum mounted at right angles to its normal anatomical position. It then occurred to me that this might be a case of Whipple's disease, and microscopic sections were at once prepared from the bowel and from an attached lymph node. These showed the presence of fat and mucoproteins in the intestinal villi and lymph-node sinuses, strong confirmatory evidence of the presumed diagnosis.

Reference to the post-mortem records of 1894 revealed, together with the necropsy report, a short medical history of the case, and there was a footnote to the effect that the case had been reported by Dr. (later Sir William) Allchin and Dr. R. G. Hebb in the *Transactions of the Pathological Society of London* (Allchin and Hebb, 1895).

The patient was a policeman named Frederick J. Adams and he was aged 38 at the time of his death. For three months he had been treated at home for diarrhoea and wasting, which was thought to be due to malignant disease. Before this he had been perfectly healthy. Following treatment for diarrhoea he improved sufficiently to be able to return to work, but 24 hours later the diarrhoea recurred, accompanied by vomiting, and he was admitted to Westminster Hospital (in those days in Broad Sanctuary facing the Abbey) on 22 August 1894 and was placed under the care of Dr. Allchin.

The clinical data are scanty. He was 'blue and collapsed' on admission; the vomit was dark green and contained numerous white flecks; the stools had a fluid, 'pea-soupy' appearance and contained large amounts of some oily substance. The urine contained bile but no albumin. Following admission he 'gradually sank' and died within 24 hours.

The necropsy was performed by Dr. Hebb. There were no external abnormalities. The proximal half of the small intestine was distended and its mucous membrane was purple and swollen but not ulcerated. In the author's words, 'the entire mucosa is beset

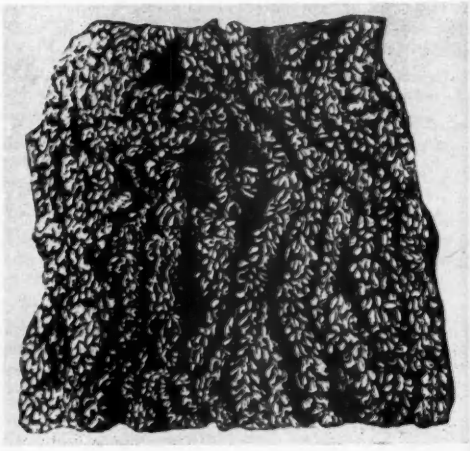

FIG. 1. *Drawing to show papillation of duodenal mucosa* (*Reproduced from* Trans. Path. Soc. London, *1895*).

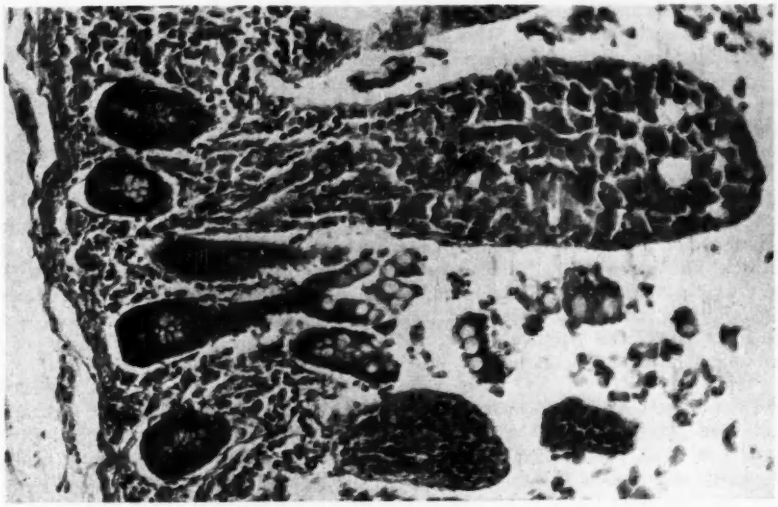

FIG. 2. *Section of duodenal mucosa from a known case of Whipple's disease. Note the papillae formed by macrophages with foamy cytoplasm and small fat spaces. Haematoxylin and eosin* × *125.*

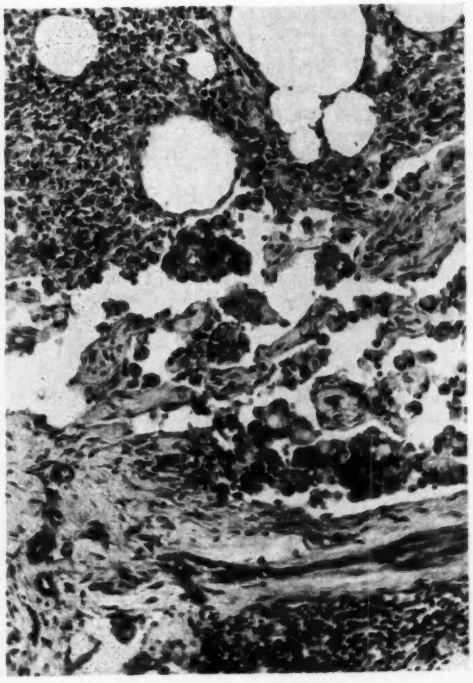

FIG. 3. *Mesenteric lymph node from Christie's case showing lymphatics distended with fat (dissolved out in section) and foamy macrophages in peripheral sinuses. Haematoxylin and eosin* × *125.*

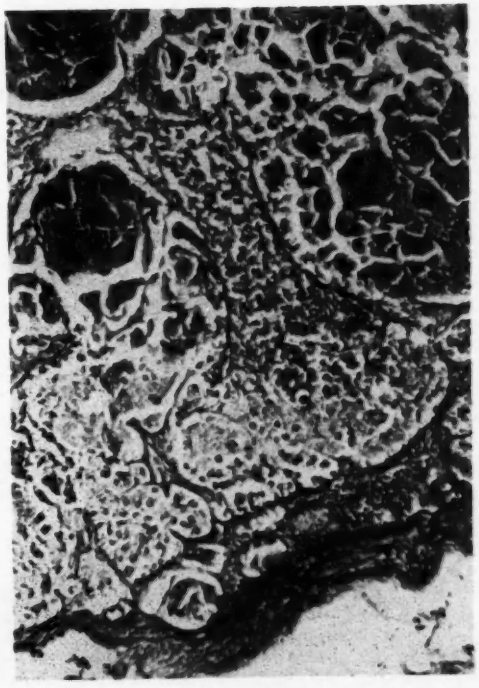

FIG. 4. *Lymph node from Allchin and Hebb's case showing broken-down macrophages in the peripheral sinuses (cf. Fig. 3) and darkly staining mucopolysaccharides in the dilated lymphatics. PAS* × *125.*

with myriads of whitish flocculi, which give it a shaggy, coarsely villous appearance'. This they illustrated with a black and white drawing (Fig. 1). The disease process was sharply limited to the small intestine, the stomach and colon being normal. It was further noted that the intestinal contents were fluid, not offensive, 'and deposited on standing a considerable whitish sediment, which on microscopical examination was found to consist chiefly of oil globules, debris, and cells'.

The mesenteric lymph nodes were 'much enlarged, tough, yet flabby' and white dilated lacteals were seen passing along the mesentery from the intestine to the glands. Beyond the glands there was 'no discoverable affection of the lymphatic system'. All the other abdominal and thoracic viscera were normal.

Histology of the intestine showed dilatation of the lymphatic vessels, the lacteals of the villi being 'distended with an amorphous, finely granular substance containing a few cells. This material only gives a slight reaction with osmic acid'. Similar appearances were noted in the lymphatics of the mesenteric nodes. All these features are illustrated by coloured drawings. The authors conclude with these words: 'The appearances seen in the villi, the lacteal vessels, and mesenteric glands, together with the absence of any apparent lesion in the lymphatic system beyond the glands, suggest a varicosity of the vessels from obstruction, but the nature and cause of the obstruction is quite obscure'.

The case was presented to the Pathological Society on 21 May 1895.

Figures 2 and 3 are photomicographs of a known case of Whipple's disease prepared from sections presented to me by Dr. A. G. Christie (Christie and Galton, 1952). They show the typical fatty globules and PAS-positive macrophages in the duodenal villi and mesenteric lymph nodes. Figure 4 is a section of lymph node prepared this year from the specimen in the Westminster museum. Despite the deterioration in cytology, the similarity to Fig. 3 is obvious, and it is interesting to note that the PAS reaction for mucopolysaccharides is strongly positive after a lapse of 66 years.

Does the fact that Whipple was anticipated in the description of his disease mean that we should henceforth call it 'Allchin's disease?' Similar claims have been made in the case of other maladies with eponymous titles, *e.g.*, Sjögren's disease, Reiter's disease, and no doubt many others—but without success. Allchin and Hebb's account is admirable in its conciseness, but that of Whipple runs to 19 columns of small print. He was not merely recording a curiosity; he was documenting for posterity, as fully as possible, a disease he believed (rightly) to be very rare and (wrongly) hitherto undescribed. It will go on being known as Whipple's disease, and we can take satisfaction in the thought that the first case to be recorded—in English at least—was described in a British journal.

I have to thank the Editor of the *Broad Way*, the organ of the students of Westminster Hospital, for permission to reproduce this article, which was originally published in the clinical supplement of March 1961.

REFERENCES

Allchin, W. H., and Hebb, R. G. (1895). Lymphangiectasis intestini. *Trans. Path. Soc. Lond.*, **46**, 221-223.

Christie, A. C., and Galton, D. A. G. (1952). A fatal case of intestinal lipodystrophy of Whipple investigated during life. *J. Path. Bact.*, **64**, 351-366.

Glynn, L. E., and Rosenheim, M. L. (1938). Mesenteric chyladenectasis with steatorrhoea and features of Addison's disease. *Ibid.*, **47**, 285-290.

Whipple, G. H. (1907). A hitherto undescribed disease characterized anatomically by deposits of fat and fatty acids in the intestinal and mesenteric lymphatic tissues. *Johns Hopk. Hosp. Bull.*, **18**, 382-391.

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An evaluation of unabsorbable markers in the study of fat absorption

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SYNOPSIS The validity of using water-soluble unabsorbable markers in the study of fat absorption was investigated.

Nine subjects were fed a finely emulsified test meal containing carbohydrate, protein, fat, and the water-soluble unabsorbable markers, polyethylene glycol, and/or phenol red. The ratio of fat to marker in recovered gastric contents was significantly different from that in the test meal. This was due to the dissociation of the meal into two phases, a solid phase rich in fat and poor in marker and a liquid phase poor in fat and rich in marker. The liquid was preferentially emptied from the stomach and also more readily sampled by a small bore tube.

When this artifact is disregarded samples obtained from the proximal small intestine would erroneously indicate an extensive and rapid absorption of fat in the duodenum and proximal jejunum.

The use of polyethylene glycol 4,000 as a non-absorbable marker in the study of the absorption of fat, carbohydrate, and protein from the small intestine of man was introduced by Borgström, Dahlquist, Lundh, and Sjövall (1957) and is being increasingly used (Lundh, 1958: Borgström, 1960; Borgström, Lindquist, and Lundh, 1961).

When investigating fat absorption with this technique a homogeneous liquid test meal is fed and samples are recovered from the intestine through a fine plastic tube during the course of digestion. The test meal contains fat and the unabsorbable marker in a known ratio. If the ratio of fat to marker in the recovered sample is less than that in the test meal fed, this is attributed to the absorption of fat from the intestine above the point of sampling and the percentage absorption may be calculated. The assumption is made that there is no dissociation between marker and fat except that caused by the absorption of fat.

Polyethylene glycol is in many ways an ideal substance to use as a non-absorbable marker (Borgström *et al.*, 1957). However, Lundh (1958) found the standard error of 235 duplicate determinations in intestinal contents to be 6·8%. In searching for a marker substance that could be more precisely estimated, phenol red, which has been used for this purpose in animal experiments, (Reynell and Spray, 1956) was investigated. Preliminary experiments showed that while between 70% and 80% of an intramuscular dose was excreted in the urine, less than 5% of an oral dose was recovered from the urine.

Before embarking on a large-scale study of fat absorption, using phenol red as a non-absorbable marker, the validity of the assumption of non-dissociation was tested by examining the ratio of fat to phenol red in the gastric contents during digestion of a test meal. This ratio should remain the same as in the test meal, since the stomach is not known to secrete or absorb fat.

A large discrepancy was found between the ratio in the stomach contents and in the test meal. The investigation was therefore extended to include polyethylene glycol as a marker and a test meal similar to the one that Borgström had used with apparent success.

METHODS

Subjects, who were medical staff or ward patients, were fasted overnight and a gastric tube passed into the body of the stomach. The position of the tube was checked fluoroscopically and the tube fastened to the face with adhesive tape to prevent entry into the duodenum. In four of the first five subjects the position of the tube was

[1]In receipt of a personal grant from the Medical Research Council.

checked at the end of the procedure. Four hundred ml. of one of the test meals (see below) was fed and samples were aspirated by syringe at the times stated. The total sample recovered was analysed in studies 1-5.

In subjects 6 and 7 attempts were made to empty the stomach two hours after the meal. This was successful in subject 7 as shown by clear samples in the water washings but impossible in subject 6 owing to blocking of the tube. In studies 8 and 9, two hours after the meal the throat was anaesthetized with an anaesthetic lozenge and complete emptying of the stomach attempted by a large bore tube. Subject 8 vomited the gastric contents, but all the contents of the stomach of subject 9 were aspirated.

The composition of the two test meals used was as follows:—

Test Meal A	Olive oil	50 g.
	Gelatin	50 g.
	Glucose	50 g.
	Phenol red	250 mg.
	Water	1,000 ml.
Test Meal B	Corn oil	74 g.
	Casilan	76 g.
	Lactose	50 g.
	Glucose	144 g.
	Polyethylene glycol	5 g.
	Phenol red	250 mg.
	Water	1,000 ml.

The test meals were homogenized using a high-pressure homogenizer.

Each sample was subjected to a two-stage extraction procedure in the system water, methanol, ether, heptane, 1:1:1:1. Three lower phases were passed through two upper phases. The separating funnels were centrifuged to ensure separation of phases. The upper phases were combined and the solvents evaporated. The residual fat was weighed. The three lower phases were combined and made up to volume. Phenol red was estimated in aliquots of this solution after centrifugation to remove solids. The optical density at 580 mμ was measured in a medium of 50% methanol saturated with potassium sulphate and made alkaline with potassium hydroxide under standard conditions. Polyethylene glycol was measured in aliquots of the lower phase after they had been evaporated almost to dryness to remove methanol and reconstituted with water. The method described by Hydén (1956) was used with the modification that the Somogy filtrates were passed through a mixed bed ion-exchange resin before the turbidimetric analysis.

Aliquots of the test meal administered were analysed with the gastric samples from each study and the 'expected' amount of fat calculated from this analysis.

RESULTS

The results from five studies in which serial samples were withdrawn from the stomach are presented in Table I. The column headed 'expected fat' gives the amount of fat one would expect to be associated with the phenol red or polyethylene glycol found in the sample if the ratio of fat to marker in the test meal were maintained in the stomach. The columns headed 'ratio fat: marker' give the ratio of fat to marker in the sample if by choice of appropriate units the ratio in the test meal is made 1. It was impossible to detect any polyethylene glycol in the 165 min. sample in study 4. In view of the limitations of the method used in the determination this can only be interpreted as indicating a very low concentration of polyethylene glycol in this sample. The phenol red concentration was also low, being only 16% of that in the 65 min. sample from this study.

The ratio of fat to marker differs from that in the test meal by more than 10% in all the samples. The

TABLE I

RELATION OF FAT TO MARKERS IN SERIAL SAMPLES FROM THE STOMACH

Subject	*Test Meal*	*Tube Internal Diameter (mm.)*	*Time (min.)*	*Sample Volume (ml.)*	*Actual Fat (mg.)*	*Expected Fat*		*Ratio Fat: Marker Test Meal = 1*	
						By Phenol Red (mg.)	*By Polyethylene Glycol (mg.)*	*Phenol Red*	*Polyethylene Glycol*
1	A	1·2	85	5	145	113		1·28	
			113	5	170	98		1.74	
			143	3	64	53		1·21	
			170	4	121	43		2·81	
2	A	3	40	5	153	125		1·22	
			70	5	82	104		0·79	
			105	5	42	67		0·63	
			130	5	25	43		0·58	
3	A	3	35	5	131	113		1·16	
			65	5	126	113		1·11	
			95	5	51	101		0·51	
			125	5	34	59		0·58	
			170	5	11	25		0·44	
4	B	3	65	5	217	145	159	1·50	1·36
			140	4·5	21	63	46	0·33	0·46
			165	9·5	73	43	[1]	0·69	[1]
5	B	3	60	5	21	53	85	0·40	0·25
			120	5	14	53	102	0·26	0·14
			180	5	62	81	91	0·77	0·67

[1] No polyethylene glycol.

TABLE II

RELATION OF FAT TO MARKERS IN ENTIRE STOMACH CONTENTS TWO HOURS AFTER FEEDING TEST MEAL B

Subject	*Tube Internal Diameter (mm.)*	*Sample Volume (ml.)*	*Expected Fat*		*Actual Fat (mg.)*	*Ratio Fat: Marker Test Meal = 1*	
			By Phenol Red (mg.)	*By Polyethylene Glycol (mg.)*		*Phenol Red*	*Polyethylene Glycol*
6	3	5 } duplicate	94	78	64	0·68	0·83
		5 }	94	78	51	0·55	0·66
7	3	5 } duplicate	72	47	84	1·19	1·75
		5 }	72	45	84	1·19	1·82
8	6 (vomited)	5 } duplicate	127	46	235	1·86	5·1
		5 }	131	46	239	1·84	5·2
9	6	5 + Filtrate Solids	99	13	170	1·72	13·1
		1 Filtrate	40	18	8	0·2	0·44
		2 Solids	101	4	238	2.36	59·5

range of ratios is 2·8 to 0·14. There is no consistent pattern. In subject 1 all the ratios are greater than in the test meal while in subject 5 all are less than in the test meal.

The appearance of the samples was varied; a few, especially the earlier ones, seemed to retain the homogenity of the test meal but most contained solid and liquid phases. It was frequently difficult to obtain a sample as the tube blocked. For these reasons it was felt that the inconsistency in the results might result from the samples obtained not being representative of the total gastric contents at the time they were taken. Studies 6-9 were done to overcome the difficulty of un-representative samples by recovering the total gastric contents two hours after feeding the test meal and homogenizing externally before taking samples for analysis. The results of these four studies are shown in Table II.

Although it is difficult to be sure of the completeness of any of these collections, study 6 is the only case where the collection was obviously incomplete due to the blockage of the tube. In study 9 the gastric content was filtered through a 1 mm. mesh wire gauze to simulate sampling through a narrow-bore tube. The solids which remained on the gauze and the fluid portion that passed through were analysed separately. The results of these analyses were combined arithmetically to calculate the composition that a 5 ml. aliquot of the total sample would have had and are presented as such as well as the analysis of the separated fractions.

Apart from study 6, where the collection was incomplete, these samples show a consistently raised fat-to-marker ratio which is especially marked for polyethylene glycol. The difference between the results obtained with the two markers probably indicated binding of the phenol red by the precipitated portion of the test meal. The separation of the solid from the liquid portion of the test meal in study 9 and the very different ratios found in the two parts lend support to the idea that the variable results in Table I are due to unrepresentative samplings.

DISCUSSION

It is obvious that the basic assumption that there is no dissociation of fat and markers in the stomach is not valid. The originally homogeneous test meal is fairly rapidly converted into a mixture of a solid phase rich in fat and poor in water-soluble marker, and a liquid phase poor in fat and rich in marker. In studies 7 to 9 the fat-to-marker ratio of the entire gastric contents recovered at two hours is higher than in the test meal. This indicates that the liquid phase leaves the stomach more rapidly than the solid, which is in agreement with the other reports on gastric digestion (James, 1957). It necessarily follows that in the portion of the test meal that has left the stomach the fat-to-marker ratio was less than in the test meal. Thus samples collected from the small intestine in this period would show apparent absorption whether in fact absorption has taken place or not.

Since studies of fat absorption from the small intestine using very similar techniques have been reported, it is important to know whether there was also a similar dissociation between fat and marker in the stomachs of the subjects used in these studies. In the absence of direct evidence of the state of the gastric contents one can only examine the reports for indications that a similar process was taking place. There are two interrelated phenomena to be taken into account: the dissociation of the test meal into solid and liquid phases with consequent dissociation of fat and marker, and the error in sampling through a small-bore tube that then arises. The first of these, coupled with the selective gastric emptying, would lead one to expect results that show a low fat-to-marker ratio in samples from the proximal small intestine during the first hours of digestion followed

by an increase in the ratio towards the end; *e.g.*, studies AI, AII, BI, and BII in a report by Borgström *et al.* (1961) show exactly this behaviour. In studies AI, AII, and BI the ratio of fat to marker of the final samples exceeded that of the test meal.

It would be expected that in spite of the selective effect of the small-bore tube for the low-ratio liquid phase occasional samples would be recovered from the small intestine with a fat:marker ratio higher than that in the test meal. The extensive discussion devoted by Lundh (1958) to this phenomenon demonstrated its occurrence in his studies also. One can only conclude that the behaviour of the test meal described in the present report was the same in the other reported studies using this technique and is probably general in this type of study. The interpretation of the results as to the site and extent of absorption of fat are of little value until it has been conclusively shown that dissociation of fat and marker, except that caused by the absorption of fat, has not taken place.

We wish to thank Mr. J. W. Hadgraft, Group Chief Pharmacist, Royal Free Hospital, for preparing the test meals.

REFERENCES

Borgström, B. (1960). Studies on intestinal cholesterol absorption in the human. *J. clin. Invest.*, **39**, 809-815.

——, Dahlquist, A., Lundh, G., and Sjövall, J. (1957). Studies of intestinal digestion and absorption in the human. *Ibid*, **36**, 1521-1536.

——, Lindquist, B., and Lundh, G. (1961). Digestive studies in children. *Amer. J. Dis. Child.*, **101**, 454-466.

Hydén, S. (1956). Kungl. Lantbrukshögskolans Annaler. [A turbidimetric method for the determination of higher polyethylene glycols in biological materials]. *Ann. roy. Agr. Coll. Sweden*, **22**, 139-145.

James, A. H. (1957). *The Physiology of Gastric Digestion.* Arnold, London.

Lundh, G. (1958). Intestinal digestion and absorption after gastrectomy. *Acta chir. scand.*, Suppl. 231.

Reynell, P. C. and Spray, G. H. (1956). The simultaneous measurement of absorption and transit in the gastro-intestinal tract of the rat. *J. Physiol.* (*Lond.*), **131**, 452-462.

Gastroenterological Society of Australia

The third annual general meeting was held at the Princess Margaret Hospital for Children, Perth, on 4 May 1961.

Office Bearers

President	Dr. W. E. King
Vice-President	Professor R. R. Andrew
Hon. Secretary	Dr. D. J. Fone
Hon. Treasurer	Dr. A. Kerr Grant

Other members of council: Sir William Morrow, Dr. S. J. M. Goulston, Dr. W. Irwin.

After the business meeting, a scientific meeting was held.

R. A. JOSKE and D. H. CURNOW outlined the history and metabolic basis of the d-xylose absorption test; the normal value of the test in an Australian population was found to be 6·6± 1·4 g./5 hr. The lower limit of normal was then arbitrarily taken as 3·8 g./5 hr. (= mean − 2SD.). Analysis of 200 xylose tests in various disease states showed that a patient with low xylose absorption usually presents other evidence of malabsorption if this is sought, although any degree of malabsorption may occur with normal xylose absorption. Xylose absorption was unrelated to fat, protein, folic acid, B_{12}, Ca, or K metabolism. The xylose test was low in atrophic jejunitis and infective enteritis, variable in post-gastrectomy syndromes, the reticuloses and vascular disease, and usually normal in pancreatic disease.

J. B. BLACKWELL described two patients with 'Malabsorption in the presence of primary carcinoma of the small bowel'. Neither had any previous history suggesting an absorptive defect.

The first case was a man, aged 72 years, who had persistently vomited for eight weeks before laparotomy at which a constricting adenocarcinoma of the mid-jejunum was excised. In the small bowel adjacent to the tumour histological changes of atrophic jejunitis were found. The villi were shortened and fused, the lamina propria inflamed, and pigment granules were prominent in the muscle layer. Clumping and segmentation were demonstrated radiologically, and biochemically a low plasma carotene, low serum albumin, low xylose absorption, and raised faecal fat confirmed malabsorption.

The second case was a man, aged 57 years, who also presented with subacute obstruction caused by an anaplastic carcinoma arising in the lower jejunum. Atrophic jejunitis with complete loss of villi was found in the proximal segment and was present, but to a lesser degree, distally. Malabsorption was considered to be present on radiological evidence and on the biochemical findings of low plasma carotene and potassium values, and low serum albumin and calcium. Faecal fat was 26%.

In a review of 12 other cases of small bowel carcinoma from the hospital files mild changes of atrophic jejunitis were found in two.

Dr. Blackwell suggested that the association of small bowel carcinoma, atrophic jejunitis, and malabsorption may be more frequent than is at present suspected.

D. W. PIPER presented a paper on 'Anticholinergic drugs and gastric secretion', in which he compared a series of new anticholinergic agents with atropine, regarding their effect on saliva flow and gastric secretion and the effect of anticholinergic drugs in general on the various constitutents of gastric juice. All anticholinergic drugs tested, including atropine, have a greater inhibitory effect on gastric secretion than they have on saliva flow. Of the drugs studied (atropine, propantheline, oxyphencyclimine, and proprionyl atropine methyl nitrate), only oxyphencyclimine and propantheline had a selective action on the stomach superior to atropine.

When the effect of anticholinergic drugs was studied on the electrolyte content of gastric juice, a progressive drop in acid output with decreasing volume of secretion was found; the acid concentration showed a less marked fall. With increasing anticholinergic suppression of secretion the sodium and potassium output fell, the potassium output falling to a greater extent than the sodium output. The potassium concentration remained relatively fixed or showed a slight fall with decreasing volume of secretion, while the sodium concentration rose. When the effect of anticholinergic drugs on the mucus content of gastric juice was studied, it was found that increasing anticholinergic suppression was accompanied by a rising mucus concentration; in many cases the total output of mucus showed little change in output with the decrease in secretory volume.

J. G. RANKIN, M. R. PLAYOUST, and R. W. BEAL described their studies on the use of P^{32}-labelled chronic phosphate for the measurement of liver blood flow. The rate, efficiency of hepatic extraction, and sites of removal of subcritical doses of colloidal P^{32}-labelled chronic phosphate from the blood stream were studied in 30 patients with normal and abnormal liver function.

Values for $t\frac{1}{2}$ were similar to those obtained by other workers, but the efficiency of hepatic extraction was found to have a roughly linear relation to the degree of hepatic dysfunction as measured by bromsulphalein retention and to range from 40% to 93%.

In the presence of normal liver function the liver and spleen together removed approximately all of the administered dose of colloid whereas in cirrhotic patients there was a relative alteration in phagocytic activity of the various organs so that the marrow removed as much as 60% and the liver and spleen together as little as 26% to 56%.

The presence of a significantly large extrasplanchnic

site of removal of subcritical doses of colloid in patients with chronic liver disease invalidates methods using these substances for measuring liver blood flow under these conditions.

A. R. COOKE and A. P. SKYRING discussed 'The use of indocyanin green as an index of hepatic function'. This was studied in a series of 93 subjects by a method requiring two blood samples exactly 10 minutes apart. A dose of 0·1 mg./kg. was used. The results were expressed as percentage retention at 10 minutes.

In a group of 20 normal subjects and 24 patients with various medical diseases but without liver disease the values were all less than 20%. In 48 patients with liver disease the values were all greater than 20% retention. There was a good correlation between severity of liver disease and percentage retention of the dye. There were no side effects. They concluded that indocyanin green is a reliable index of liver function and that values greater than 20% by this method represent evidence of liver disease.

R. W. BEAL and C. R. B. BLACKBURN discussed 'Studies on blood ammonia levels in patients with liver disease', with reference to the effect of nialamide. Nialamid (Niamid, Pfizer) has been shown to lower the level of blood ammonia in patients with cirrhosis of the liver, whether the elevation is endogenously or exogenously produced. Hepatic vein and renal vein catheterization, together with forearm and liver blood flow measurements were made to determine the mode of action. It was postulated from these results that Niamid may act by increasing hepatic blood flow, while maintaining a constant peripheral uptake, the net effect being to present more circulating ammonia to the liver for detoxication.

Observations on the 'Diagnosis and treatment of haemochromatosis' were presented by W. B. HENNESSY and BRUCE HALL. Thirty cases of haemochromatosis were studied. Twenty-one were men and nine were women. The age at which the disease was diagnosed varied from 19 to 80 years, with a peak incidence in the sixth decade. Two patients had a family history of haemochromatosis, three had suffered from severe anaemia, and five had a history of alcoholism. Hepatomegaly occurred in every patient; skin pigmentation was noted in 22 of these but diabetes mellitus occurred in only eight. The diagnosis was confirmed by either histological examination of the liver or serum iron studies or both. Gastric biopsy was performed in nine patients, and in all of these haemosiderin was demonstrated in the chief cells of the tubular glands.

Seventeen patients were treated by repeated venesection and in six the haemoglobin or serum iron levels eventually fell. To accomplish this, from 10g. to 26g. iron was removed.

Half the patients died within the follow-up period, but only four of these had been subjected to venesection. Five died from primary carcinoma of the liver.

All the surviving patients have experienced increased strength and well-being after phlebotomy. Skin pigmentation has lessened and the liver has become smaller. Liver function tests have improved. Serial liver biopsies showed diminution or complete disappearance of iron but the cirrhosis was unchanged.

B. F. VAUGHAN and W. R. PITNEY described the 'Radiological findings in 63 cases of megaloblastic anaemia'. They had reviewed the barium meal films in these cases without reference to the haematological findings.

Haematological diagnosis depended on the results of serum vitamin B_{12} concentrations, radioactive vitamin B_{12} absorption tests, and gastric biopsy as well as the more usual diagnostic techniques and the response to therapy. Radiological gastric atrophy was demonstrated in 42 patients, 38 with pernicious anaemia and four with the malabsorption syndrome. All 42 patients suffered from vitamin B_{12} deficiency.

In eight patients, megaloblastic anaemia occurred following gastric surgery, making a total of 50 abnormal barium meal examinations in the 63 patients. In one patient with folic acid deficiency megaloblastic anaemia due to malabsorption, in five with nutritional megaloblastic anaemia, and in two with megaloblastic anaemia associated with lactation, the barium meal findings were normal. In five patients with pernicious anaemia a normal barium meal was reported.

Sixteen of the 42 patients with radiological gastric atrophy demonstrated oesophageal regurgitation, six with associated hiatal hernia. The demonstration of a gastric abnormality in pernicious anaemia was fairly accurate (90%); radiological examination was unable to assist in the identification of the mechanism of the anaemia in patients with a radiologically normal stomach or with partial gastrectomy.

PETER PARSONS reviewed a series of 16 patients with 'Gas cysts of the colon'. Their ages ranged from 30 to 70 years, and there were twice as many women. The left side of the colon was the most common site, particularly the splenic flexure and sigmoid.

All but three suffered from chronic bronchial asthma and at least 11 of these had been treated with corticosteroids for from one to five years. Ten cases presented with diarrhoea, the stools usually containing mucus, but appreciable bleeding occurred in three. One had subacute obstruction. When the cysts were low the diagnosis was established by sigmoidoscopy, but mistakes were made in four early cases before the observer was aware of the rather characteristic appearances. The higher cysts were detected by radiographs, the plain film showing the foam-like collections of gas shadows in the line of the colon in five of the cases in which they were done, and the barium enema gave confirmation in every case, but here again was misinterpreted in four of the early cases.

'Combined early endoscopy and radiology in the management of upper intestinal bleeding' was discussed by A. R. COOKE and A. P. SKYRING.

Forty-five consecutive and unselected patients admitted with upper intestinal bleeding were studied using a combination of early endoscopy and radiology. Endoscopy was performed in the ward within 24 hours of admission and was followed by a barium or gastrografin meal in the Department of Radiology. The early diagnosis was

confirmed at necropsy, operation, or a routine barium meal before discharge from hospital. An overall diagnosis rate of 82% was achieved within the first 24 hours. All gastric ulcers were detected by this method but duodenal ulcers were missed on two occasions.

Acute gastric erosions were seen in 10 patients. There remained a group of six patients in whom no diagnosis was made by either early or late investigation. The procedures were safe and in all cases an excellent view could be obtained with the gastroscope. It is hoped that early diagnosis places further management, especially if surgery is contemplated on a safer and surer clinical basis.

BRUCE BEVERIDGE described a 'Comparative study of various liver function tests in the differential diagnosis of jaundice'. A retrospective diagnosis was made on clinical, epidemiological, histological, and follow-up evidence in 53 patients with clinical jaundice, divided into five diagnostic groups: Extrahepatic obstruction, cholangiolitic hepatitis, parenchymal jaundice, drug cholestasis, and chronic liver disease. For each liver function test a scattergram showed the values obtained in the five diagnostic groups and from these the reliability of the test in differentiating the groups was judged.

Clinically jaundice due to chronic liver disease and drug-induced cholestasis rarely caused grave diagnostic problems, but the diagnosis of extrahepatic obstructive jaundice from cholangiolitic hepatitis and occasionally from parenchymal jaundice presented difficulty.

The serum alkaline phosphatase activity was not found to be a good indicator of extrahepatic obstruction. The most useful indicator of parenchymal jaundice was a thymol turbidity value greater than 3 units. Less valuable were markedly raised serum oxalo-acetic and pyruvic transaminase levels, while serum ornithine transcarbamylase and quinine oxidase levels were of little value. However, on biochemical grounds five patients appeared to have extrahepatic obstruction, though this was not in fact present.

The thymol turbidity results appeared independent of serum albumin and globulin concentration, which were helpful only in chronic liver disease.

The September 1961 Issue

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Primary Crohn's disease of the colon and rectum J. S. CORNES and METTE STECHER

Difficulties in the diagnosis and management of unsuspected tuberculous enteritis and colitis E. J. MORAN CAMPBELL

Recurrent swelling of the parotid gland R. S. BRUCE PEARSON

$Co^{58}B_{12}$ absorption (hepatic surface count) after gastrectomy, ileal resection, and in coeliac disorders D. J. FONE, W. T. COOKE, M. J. MEYNELL, and E. L. HARRIS

Comparative investigations on the activity of leucine aminopeptidase, glutamic oxalacetic transaminase, and alkaline phosphatase in serum B. G. MUNCK and K. KJERULF

The effect of anticholinergic drugs on the electrolyte content of gastric juice D. W. PIPER and MIRJAM C. STIEL

Non-esterified fatty acids and lipoprotein lipase activity in patients with cirrhosis of the liver C. CHLOUVERAKIS and PETER HARRIS

The effect of vagotomy and pyloroplasty on the maximal acid response to histamine ALVIN M. GELB, IVAN D. BARONOFSKY, and HENRY D. JANOWITZ

The measurement of intestinal sounds in man and their relationship to serum 5-hydroxytryptamine B. G. ADAMS

The cardiac sphincter in the cat C. G. CLARK and J. R. VANE

Serum studies in man after administration of vitamin A acetate and vitamin A alcohol OLIVER FITZGERALD, JAMES J. FENNELLY, and DANIEL J. HINGERTY

The problems of closed liver injuries R. H. B. MILLS

Methods and techniques

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